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FRONTIERS IN ANIMAL SCIENCE – HIGHLIGHTS FROM ITS FIRST YEAR

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ORIGINAL RESEARCH
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Economic Cost of Traceability in U.S. Beef Production

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Livestock traceability has increasingly become a focus for the USDA, the National Cattlemen's Beef Association, high-volume beef-exporting states, and other beef industry stakeholders. The focus on traceability within the United States (U.S.) began after several international animal disease outbreaks and continues to be of importance with highly infectious diseases spreading across the globe. Mitigating adverse future disease outbreaks and food safety events, as well as maintaining export markets through a positive international perception of U.S. beef has become a top priority. Implementing a national animal identification (ID) and traceability program would enable the industry to track and reduce the potential losses due to an outbreak or event. However, such a system comes at a cost, mainly to cow-calf producers. This study utilizes a partial equilibrium model to determine the impacts of a beef cattle animal ID and traceability system in the United States. Utilizing an economic model allows us to provide a comparison of how the various beef sectors would need to respond to offset the costs of a national animal ID and traceability program. Assuming no changes in domestic and international demand for U.S. beef, producers at the wholesale, slaughter, and feeder levels lose $475 million, $1,143 million, and $1,291 million, respectively, in a 10-year discounted cumulative producer surplus. A 17.7 and 1.9% increase in international and domestic beef demand would be required to completely offset the producer costs of CattleTrace, respectively.

Keywords: animal identification, beef production, partial equilibrium model, traceability, welfare analysis

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INTRODUCTION

The United States (U.S.) is relatively "behind" other countries in implementing a national animal identification (ID) and traceability program. Other large beef exporters, including Argentina, Australia, Canada, European Union, New Zealand, and Uruguay, all have government mandated systems (Schroeder and Tonsor, 2012). Despite the lack of a national animal ID and traceability program, U.S. beef has remained internationally competitive and generally accepted as a safe source. This, along with fear of increased cost and other long-term implications, has led some industry stakeholders to disapprove of potential government-mandated animal ID and traceability programs (Golan et al., 2004).

Beef production in the United States is highly segmented, which causes livestock to have several changes of ownership between birth and slaugher. The principal product of cow-calf operations is weaned calves, which are subsequently sold to stockers, backgrounders, or feedlots. Some calves from cow-calf operations are transferred directly to feedlots at, or around, the time of weaning, in which case, they are referred to as "calf-feds" that remain in the feedlot prior to being harvested

(Drouillard, 2018). The largest share of the calf population, usually 60% or more, is first placed into a backgrounding or stocker operation, or a combination thereof (USDA, 2018). Most cattle pass through a feedlot at some point before reaching slaughter. The segmentation, production differences, and geographical disbursement further complicates the tracing and tracking system.

In addition, there are over 103 million head of cattle in the United States, with 192,000 head and over 2.6 billion pounds of beef exported in 2018 (USDA-NASS, 2018a,b). This high volume of production and global demand for U.S. beef complicates the ability to trace, or physically track a product, through the typical U.S. beef supply chain.

Several studies, including Coffey et al. (2005), have assigned an opportunity cost to the expected impact of a disease outbreak, specifically BSE (bovine spongiform encephalopathy), in the United States. These studies support the positive impact that a traceability program could have on the U.S. beef industry in avoiding lost export markets and loss of inventory. Measuring the potential impacts of an outbreak has been considered from many different perspectives and all suggest a significant negative impact to the industry; so much so that the National Cattlemen's Beef Association included traceability in their Long-Range Plan for 2016–2020 (National Cattlemen's Beef Association, 2017). However, determining the true costs and impacts of a traceability program within the United States is difficult due to the nature of the U.S. supply chain, but is crucial as a national animal ID and traceability program is eminent. Understanding the potential economic impact of a traceability program is important, especially in a large beef producing state such as Kansas. In addition, it is important to recognize which segments of the industry may be affected the most.

The objective of this study is to analyze the economic impacts of an animal ID and traceability system. Specifically, we calculate the direct costs of implementing an animal ID and traceability system, called CattleTrace, for each segment in the U.S. beef industry. Next, we incorporated the cost estimates into a partial equilibrium model of the U.S. livestock and meat industry to determine the short- and long-run economic impacts to the various segments of the U.S. beef industry.

In 2018, a pilot program, called CattleTrace, was launched with the support of industry stakeholders to begin directing the beef industry toward a cohesive traceability program. In January 2020, a new initiative, U.S. CattleTrace, combined the efforts of CattleTrace, which includes multiple partners, including the Kansas Livestock Association and others in Kansas, Missouri, Oklahoma, Kentucky, Oregon, and Washington, as well as traceability pilot projects underway in Florida and Texas (U.S. CattleTrace, 2020). The CattleTrace program extends from beginning-to-end of the beef industry and includes participants from all segments of production. Current participation from beef industry stakeholders includes many cow-calf producers, 12 livestock markets, 2 backgrounders, 16 feedlots, and 3 major packers (4 locations). While the CattleTrace program began in Kansas, multiple states are now part of the system with various private and public organizations establishing partnerships in an effort to illustrate how a national animal ID and traceability program may look in the future. The following analysis is based on the cost estimation of implementing a national animal ID and traceability program structured as CattleTrace.

CattleTrace is a voluntary program where cattle producers from all segments (cow/calf, backgrounders and stockers, sale barns, feedlots, and packers) can select to participate. At the cow/calf level, producers must implement the use of UHF identification for all calves leaving the premises, and depending on size of the operation (economies of scale) either a wand tag reader or panel reader is required. Increased labor costs, tag costs, and reader costs are all taken into consideration as well as a large body of assumptions on animal injury, human injury, and more. Backgrounders and stockers are assumed to need replacement tags as well as readers (wand or panel depending on economics of scale) to meet the CattleTrace requirements. Sale barns will require panel readers, with a higher quantity of panels required for larger facilities (economies of scale assumption). Feedyards are also expected to implement the use of panel readers and replacement tags if needed. All of these segments of the industry cost estimations make assumptions about labor requirements, injuries, and more based on an extensive literature review and industry discussion. At the packer level, software implementation and panel readers are the biggest initial costs for the segment. All data is stored and managed by CattleTrace in a secure location.

DATA AND METHODS

An annual multi-market partial equilibrium model of the U.S. livestock and meat industry was employed to estimate the economic impacts of industry costs incurred through the adoption of CattleTrace. In general, as additional costs are incurred throughout a vertically-related marketing chain, livestock, and meat prices and quantities are impacted. Furthermore, changes in prices at the retail level for beef will influence the demand for substitute products (e.g., pork, poultry, and lamb). A traceability system could also positively influence domestic and international demand for U.S. beef. However, the extent of these potential changes is difficult to forecast.

The economic model utilized in this study is an updated version of the multi-market partial equilibrium model documented in Pendell et al. (2010), Pendell et al. (2013) and Dennis et al. (2018). An equilibrium displacement model (EDM) allows for the estimation of the potential impact of a particular shock on the market, in this case, how implementing an animal ID and traceability program will impact prices and quantities on the livestock and meat markets. Such a model allows for changes in both supply and demand across multiple markets, in this case between beef, pork, poultry, and lamb.

The EDM contains an underlying set of structural demand and supply functions. After totally differentiating the structural demand and supply functions and converting to an elasticity form, the result is an EDM of the U.S. livestock and meat industry (see **Appendix** Equations A1–A30). After solving for the changes in the endogenous prices and quantities, changes in consumer

TABLE 1 | U.S. prices and quantities used in analysis, 2018.

Level	Price $/lb.	Quantity Billion lbs.
Retail beef	5.923	18.759
Wholesale beef	2.140	26.948
Imported wholesale beef	1.875	2.999
Exported wholesale beef	2.140	3.155
Slaughter cattle	1.167	44.578
Feeder cattle	1.469	36.403
Retail pork	3.745	16.632
Wholesale pork	0.752	26.315
Imported wholesale PORK	1.605	1.042
Exported wholesale pork	0.752	5.870
Slaughter hogs	0.461	35.246
Domestic retail lamb	8.204	0.132
Imported retail lamb	10.386	0.236
Wholesale lamb	3.760	0.153
Slaughter lamb	1.271	0.307
Feeder lamb	1.775	0.243
Retail poultry	1.818	35.368
Wholesale poultry	0.957	49.016
Exported retail poultry	0.957	7.763

TABLE 2 | CattleTrace direct costs to industry and supply shocks used in partial equilibrium model.

Sector	CattleTrace direct cost[a]	% of total industry cost[a]
Cow/Calf	$129,823,537	84.3
Backgrounder	$7,670,839	5.0
Sale barn	$6,439,428	4.2
Feedlot	$9,640,589	6.3
Packer	$512,936	0.3
Total	$154,087,329	100
Partial equilibrium model sector	**CattleTrace direct cost**	**% Change in supply**
Feeder cattle[b]	$136,262,965	−0.2548
Slaughter cattle[c]	$17,311,428	−0.0333
Wholesale[d]	$512,936	−0.0009

[a] *Shear et al. (2019).*
[b] *Combined Cow/Calf and Sale Barn sectors.*
[c] *Combined Backgrounder and Feedlot sectors.*
[d] *Packer sector.*

and producer surplus can be calculated using equations (1, 2), respectively:

$$\Delta CS_k = -P_k^r Q_k^r (EP_k^r - z_k^r)(1 + 0.5EQ_k^r) \tag{1}$$

$$\Delta PS_k^i = P_k^i Q_k^i (EP_k^i + w_k^i)(1 + 0.5EQ_k^i) \tag{2}$$

CS and *PS* are defined as the consumer surplus and producer surplus, respectively. *P* and *Q* indicate price and quantity, respectively. *E* represents a relative change operator. *z* and *w* are the exogenous demand and supply shifters, respectively. The superscript *i* denotes the market level [r = retail, w = wholesale (processor/packer), s = slaughter (feeding), and f = feeder (farm)] and subscript *k* denotes the species (B = beef, K = pork, L = lamb, and Y = poultry).

The exogenous shock to the EDM model is the implementation of CattleTrace. The cost of implementing CattleTrace was estimated for each segment of the industry and took into account economies of scale. The EDM model also relies on given elasticity estimates to properly estimate how the markets will respond to changes in supply and demand. The base year price and quantity data are from 2018 and reported in **Table 1** (Livestock Marketing Information Center, 2019). **Table A1** provides the remaining market parameters, including the supply and demand elasticities for the different commodities across the various sectors, which were retained as defined in Pendell et al. (2010) and Dennis et al. (2018).

The five segments of the U.S. beef industry in this study include: cow/calf, backgrounder/stocker, sale barn, feedlot, and packer. The total cost estimates for each segment are $129.82 million (cow/calf), $7.67 million (backgrounder/stocker), $6.44 million (sale barn), $9.64 million (feedlot), and $0.51 million (packer) (Shear et al., 2019; **Table 2**). The five group subtotals were summed to obtain the annual total cost for the entire beef cattle industry of adopting CattleTrace, $154.09 million. Costs associated with the cow/calf and sale barns sectors are aggregated in the feeder cattle sector, backgrounder and feedlots are aggregated in the slaughter cattle sector, and packer costs are referred to as wholesale costs in this economic analysis.

The annual beef industry CattleTrace costs are distributed as: $0.51 million to the wholesale beef sector, $17.31 million to the slaughter cattle production sector and $136.26 million to the farm sector (**Table 2**). Using 2018 average prices and quantities for each market level, these cost estimates represent the following percentage increases in CattleTrace costs relative to total value at each sector: 0.0009% at the wholesale beef level, 0.0333% at the slaughter cattle level, and 0.2548% at the farm level (**Table 2**). The percentage changes in costs at each market level are estimated in a similar manner for all scenarios.

Scenarios

Four scenarios are considered when quantifying the economic impacts of CattleTrace. The first two scenarios differ in the proportion of costs borne by the producer. The final two scenarios focus on U.S. beef demand responses by domestic and international consumers. It is assumed that 100% of producers would adopt CattleTrace.

The scenarios are separated into four areas:

1) *Effects of CattleTrace Costs with No Benefits*

The impacts of increased costs resulting from CattleTrace are simulated. This simulation assumes both domestic and international consumer demand for U.S. beef is unaffected by CattleTrace. In other words, we estimate the impacts of the costs associated with 100% adoption of CattleTrace assuming that no benefits accrue to the U.S. beef industry.

2) *Effects of a Government Cost-Share of CattleTrace Costs with No Benefits*

According to recent research by Mitchell et al. (2019), "results show that policies would be most effective at reducing costs at the cow-calf level or offering cost-shares for feedlot producers who want to procure cattle with electronic traceability." Similar to Scenario 1, we simulate the impacts of increased costs resulting from CattleTrace. However, we assume 1/3 of the costs for RFID ear tags and electronic readers are borne by the producer while the government is responsible for the remaining 2/3 of those costs. Like Scenario 1, this simulation assumes both domestic and international consumer demand for U.S. beef is unaffected by CattleTrace. In essence, we measure how a government cost-share program for CattleTrace would impact the U.S. beef industry.

3) *Increases in International Beef Demand Needed to Offset CattleTrace Costs*

Adoption of CattleTrace, or any other animal identification and traceability system, could increase foreign consumer confidence in the U.S. beef system. We estimate the increase in U.S. beef export demand (assuming constant domestic demand) that would be needed to offset producer costs of CattleTrace adoption costs.

4) *Increases in Domestic Beef Demand Needed to Offset CattleTrace Costs*

Similar to Scenario 3, we estimate how much of a domestic beef demand enhancement would be required (assuming constant export demand) to offset producer costs of CattleTrace adoption costs.

TABLE 3 | Changes in producer and consumer surplus resulting from adopting cattletrace with no benefits (million $).

Surplus measure	Short-Run[a]	Long-Run[b]	Cumulative present value
Producer surplus			
Retail beef	116.32	−0.43	11.76
Wholesale beef	−55.96	−3.54	−475.12
Slaughter cattle	−271.74	−11.37	−1, 143.13
Feeder cattle	−238.04	−41.70	−1, 291.02
Retail pork	33.90	0.05	75.53
Wholesale pork	11.06	0.03	27.65
Slaughter hog	6.32	0.03	17.28
Retail domestic lamb	0.45	0.00	0.95
Wholesale lamb	0.06	0.00	0.16
Slaughter lamb	0.02	0.00	0.09
Feeder lamb	0.02	0.00	0.09
Retail poultry	167.33	0.02	294.30
Wholesale poultry	73.57	0.02	151.19
Consumer surplus			
Retail beef	−445.01	−3.38	−1, 305.12
Retail pork	14.21	0.15	48.60
Retail domestic lamb	−0.11	0.00	−0.12
Retail imported lamb	1.09	0.00	2.37
Retail poultry	119.39	0.51	371.78

Surplus is calculated using average 2018 prices and quantities for livestock and meat.
[a] *Totals are not identical to sums of individual surpluses because they are medians of simulations.*
[b] *Short-run is year 1 and long-run is year 10.*

RESULTS

Effects of CattleTrace Costs With No Benefits

As expected, changes in prices and quantities for the U.S. beef industry were much larger when compared to the pork, poultry, and lamb industries. This is because the U.S. beef industry is the only industry with an increase in costs as a result of CattleTrace. All changes in prices and quantities within the beef industry are consistent with an increase in CattleTrace costs at the wholesale, slaughter and farm levels. An increase in costs at the farm, slaughter, and wholesale levels shifts both the primary and derived supply functions, as well as, derived demand functions at the slaughter and farm levels. This results in retail and wholesale level beef prices to increase by 0.43 and 0.42%, respectively, while quantities decline by 0.16 and 0.41%. Imported and exported wholesale beef, slaughter, and feeder cattle prices and quantities all decline. Slaughter cattle prices and quantities decline by 0.15 and 0.34%, respectively, while feeder cattle prices and quantities fall by 0.08 and 0.26%. Pork, poultry and lamb prices and quantities all increase, except for export quantities, by a small amount, as consumers substitute away from beef to relatively cheaper protein sources in response to increased retail beef prices.

Table 3 presents producer surplus impacts due to the costs implementing CattleTrace. As expected, the short-run impacts (year 1) are much larger than the long-run impacts (year 10) as producers are able to adjust to these changes in the long-run. In the short-run, the slaughter and feeder cattle sectors experience the largest losses at $271.7 and $238.0 million, respectively. The wholesale level loses $56.0 million. In the long-run, the feeder and slaughter cattle sectors lose $41.7 and $11.4 million, respectively, while the wholesale level lose $3.5 million in producer surplus. The cumulative discounted present value of producer surplus losses over 10-years for the feeder cattle, slaughter cattle and wholesale beef sectors are $1,291 million, $1,143 million and $475 million, respectively.

Effects of a Government Cost Share of CattleTrace Costs With No Benefits

Results are similar to the scenario when producers bear all CattleTrace costs, except the impacts are smaller in magnitude. This results in retail and wholesale level beef prices to increase by 0.27 and 0.27%, respectively, while quantities decline by 0.10 and 0.26%. Imported and exported wholesale beef, slaughter, and feeder cattle prices and quantities all decline. Slaughter cattle price and quantity fall by 0.10 and 0.22%, respectively, while feeder cattle price and quantity fall by 0.07 and 0.16%. Pork,

TABLE 4 | Changes in producer and consumer surplus resulting from a government cost share of cattletrace costs (million $).

Surplus measure	Short-Run[a]	Long-Run[b]	Cumulative present value
Producer surplus			
Retail beef	74.56	−0.28	7.44
Wholesale beef	−35.46	−2.32	−304.39
Slaughter cattle	−173.61	−7.40	−732.50
Feeder cattle	−154.10	−25.46	−813.39
Retail pork	21.59	0.03	48.33
Wholesale pork	7.06	0.02	17.59
Slaughter hog	4.03	0.02	11.05
Retail domestic lamb	0.28	0.00	0.60
Wholesale lamb	0.04	0.00	0.10
Slaughter lamb	0.02	0.00	0.05
Feeder lamb	0.01	0.00	0.06
Retail poultry	106.47	0.01	187.71
Wholesale poultry	46.84	0.01	96.14
Consumer surplus			
Retail beef	−283.25	−2.21	−835.56
Retail pork	9.13	0.10	31.23
Retail domestic lamb	−0.07	0.00	−0.08
Retail imported lamb	0.70	0.00	1.51
Retail poultry	76.31	0.34	237.42

Surplus is calculated using average 2018 prices and quantities for livestock and meat.
[a]Totals are not identical to sums of individual surpluses because they are medians of simulations.
[b]Short-run is year 1 and long-run is year 10.

poultry, and lamb prices and quantities all increase, except for export quantities, by a small amount, as consumers substitute away from beef to relatively cheaper protein sources in response to increased retail beef prices.

Table 4 presents producer surplus impacts due to the costs implementing CattleTrace. Similar to the previous scenario, the short-run impacts are much larger than the long-run impacts. In the short-run, the slaughter and feeder cattle sectors experience the largest losses at $173.6 and $154.1 million, respectively, while the wholesale level lose $35.5 million. In the long-run, the feeder and slaughter cattle sectors lose $7.4 and $25.5 million, respectively, while the wholesale level lost $2.3 million in producer surplus. The cumulative discounted present value of producer surplus losses over 10-years for the feeder cattle, slaughter cattle and wholesale beef sectors are $813 million, $733 million, and $304 million, respectively.

Increases in International Beef Demand Needed to Offset CattleTrace Costs

As most major exporting countries have traceability systems, implementing a national traceability program could open new markets or allow for quicker entry back into the market after a disease outbreak. This scenario was performed to determine the increase in international beef demand needed so that the U.S. beef producer sectors do not lose any producer surplus. A permanent 17.7% increase (equivalent to 558 million lbs.) in international demand for U.S. beef would be needed such that producers do not lose any surplus. To put this value into perspective, the quantity of U.S. beef exports varied from an increase of 21 to a 12% decrease between 2009 and 2018 (LMIC 2019). Furthermore, 28% (885 million lbs.), 20% (638 million lbs.), and 14% (449 million lbs.) of U.S. beef exports went to Japan, South Korea, and Mexico, respectively, in 2018 (Livestock Marketing Information Center, 2019). Thus, maintaining market access to a single export market could completely offset U.S. beef producer costs of CattleTrace.

Increases in Domestic Beef Demand Needed to Offset CattleTrace

As demand for transparency by U.S. consumers continues to increase, implementing a national animal ID and traceability program could potentially have a positive impact on consumer demand for beef. This scenario was performed to determine the increase in domestic beef demand needed so that the U.S. beef producer sectors do not lose any producer surplus. A permanent 1.9% increase (or 356 million lbs.) in domestic demand for U.S. beef would be needed such that producers do not lose any surplus. Between 2009 and 2018, annual domestic retail beef demand, on average, varied between an increase of 4.14% to a decrease of 4.10% from the previous year. Thus, a modest increase in domestic consumer demand for beef needed to offset the costs of CattleTrace has been experienced recently.

When considering economies of scale, the cost of implementing CattleTrace ranged from $2.84 to $6.06/head for cow/calf producers. For backgrounders, the cost of implementing CattleTrace ranged from $0.41 to $0.83/head. The average cost for sale barns was $0.14/head, and the cost of implementing CattleTrace for feedlots ranged from $0.33 to $0.55/head. The average cost to packers ranged from $0.02 to $0.18/head. The overall direct cost to the beef industry was estimated to be $154.09 million.

A partial equilibrium model of the U.S. livestock and meat sector was used to evaluate the impacts of adopting CattleTrace on producers. Assuming no changes in domestic and international demand for U.S. beef, producers at the wholesale, slaughter, and feeder levels lose $475 million, $1,143 million, and $1,291 million, respectively, in 10-year discounted cumulative producer surplus. If a government cost share program is implemented (i.e., 1/3 of the costs of ear tags and readers are borne by the producers while the other 2/3 of the costs are borne by the government), the producers losses are smaller; feeder, slaughter and wholesale levels lose $813 million, $733 million, and $304 million, respectively. With a possibility of increasing consumer demand as a result of traceability, two simulations evaluated the increase in international and domestic demand required to offset the costs of CattleTrace to U.S. cattle producers. A 17.7% and 1.9% increase in international and domestic beef demand would be required to completely offset the producer costs of CattleTrace, respectively.

CONCLUSION

This analysis is an overview of the costs and economic impacts of implementing CattleTrace, a ultra-high frequency based radio frequency identification (UHF-RFID) technology-based traceability program. The main objectives of this analysis was to provide an estimate of the direct cost to the industry for implementing CattleTrace, as well as, estimate the economic impact of a national animal identification (ID) and traceability program for the beef industry. Determining the direct costs to the industry required estimating costs within each industry sector.

This analysis suggests that your 'typical' small fluctuations in domestic and international beef demand on average could offset the direct costs to producers and the industry as a whole. These results may encourage more industry support for a national animal ID and traceability program; however some concerns, such as data management, cannot be addressed in this model and, therefore, remain as hurdles to the implementation of a national program.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

HS and DP contributed to the conception and design of the study. HS compiled a rough draft of the manuscript, incorporating the results section compiled by DP. Both authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2020.552386/full#supplementary-material

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The Effects of Dairy Heifer Age at Training on Rate of Learning and Retention of Learning in a Virtual Fencing Feed Attractant Trial

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A better understanding of factors that influence learning of cattle with respect to new virtual fencing technology is required to inform the development of best practice training protocols and guide the introduction of the technology to naïve dairy cattle. This experiment examined the effect of age on (1) the efficiency of associative pairing of audio and electrical stimuli in dairy heifers and (2) the retention of this associative pairing over a long period of time without use. Fifty-nine Holstein dairy heifers were used in feed attractant trials where audio cues and electrical stimuli were delivered through manually controlled training collars. Heifers were allocated to four treatments that differed in the age at which naïve animals underwent training; these were 6-months ("6M"; $n = 15$), 9-months ("9M"; $n = 15$), 12-months ("12M"; $n = 15$), or 22-months of age ("22M"; $n = 14$). Animals in the 6, 9, and 12M treatments underwent a second round of training at 22-months of age (i.e., at the same time as naïve 22M heifers). Heifers received an audio stimulus (2 s; 84 dB) when they breached a virtual fence after which a short electrical stimulus (0.5 s; 3 V, 120 mW) was administered if they continued to move forward. If the animal stopped moving forward no further stimuli were applied. There were no effects of age treatment on the total number of interactions with the virtual fence ($P > 0.05$). During initial training, 22M heifers received a lower proportion of electrical stimuli (i.e., responded to audio without requiring the electrical stimulus; $P < 0.001$) and more frequently stopped walking ($P = 0.01$) and turned back ($P = 0.008$) following administration of the audio cue compared to younger heifers. Previous training at an early age did not improve the responsiveness of heifers to virtual fencing when re-trained at 22-months of age ($P > 0.05$). We conclude that dairy heifers should be trained to virtual fencing technology close to calving age rather than earlier in their ontogeny and that stock be re-trained following an extended period without virtual fencing technology.

Keywords: associative learning, conditioning, development, sensitive period, shock

INTRODUCTION

Virtual fencing is an emerging technology that has the potential to reduce labor and fencing costs and facilitate more intense or complex grazing regimes in pasture-based dairy systems. Animals are trained to associate an audio cue that is delivered via a neckband mounted device as they approach a boundary set via global positioning system with a pending electrical stimulus, unless they stop moving toward the virtual boundary (e.g., Campbell et al., 2019; Lomax et al., 2019). Acute stress is expected when animals are undergoing this type of avoidance learning, but this learning is essential to successful operation of virtual fencing technology (Lee et al., 2018). The stress response should be minimal once animals have learnt to avoid the electrical stimulus which restores predictability and controllability to their environment (Lee et al., 2018). Efficient and rapid learning of the association between audio and electrical stimuli may minimize the duration and intensity of the acute stress experienced during training to virtual fencing technology. A better understanding of the factors that influence associative learning of the pairing of the audio and electrical stimuli in cattle is required to inform the development of best practice training protocols and guide the introduction of virtual fencing technology to naïve dairy cattle.

Age at training is one factor that may affect the rate of associative learning. For example, Kovalčik and Kovalčik (1986) found that 15-month old heifers were more efficient than primiparous and multiparous cows at learning the location of food in a maze. Theoretical modeling identifies several periods of increased neural plasticity during ontogeny, known as "sensitive periods" (Taborsky, 2017). The brain is highly sensitive to environmental stimuli during these periods which enables rapid learning (Sokolowski and Levine, 2010). To be clear, sensitive periods do not sharply define phases during which learning can occur and outside of which it cannot, but rather a gradual change in the ease or probability of learning is observed around these phases of development (Hinde, 1970). Sensitive periods often coincide with times of rapid morphogenesis, metamorphosis, sexual maturation, or other stages of ontogeny when physiological or morphological systems are undergoing major reorganization (Stamps and Groothuis, 2010). We thus hypothesized that training dairy heifers to virtual fencing technology at periods of their ontogeny that coincide with physiological or morphological change (i.e., pre-puberty juvenile period, around puberty early adolescence period, post-puberty late adolescence period) would increase the rate of associative learning compared to training more developed heifers that are close to calving age.

It is unknown whether heifers trained to virtual fencing technology at a younger age would retain their associative learnings over long periods of time without use, ensuring more rapid adaptation when managed with virtual fencing as an adult. There is a paucity in the scientific literature regarding the long-term memory abilities of cattle. Hirata et al. (2016) found Japanese Black cows were able to retain the memory of a complex maze configuration for 6 weeks, while Kovalčik and Kovalčik (1986) observed that 77% of cows but only 46% of heifers remembered the location of food in a maze after 6 weeks. In other livestock, Lee et al. (2006) found sheep to retain the memory of a maze configuration after 6 weeks. Considering that experiences during sensitive periods can produce long lasting neurobiological and behavioral change (Sokolowski and Levine, 2010), our secondary hypothesis was that the heifers that received training to virtual fencing technology early in their ontogeny would retain these learnings and show improved responsiveness to the technology when re-trained around calving age, compared to naïve heifers that are also around calving age.

Using dairy heifers in a feed attractant trial, this experiment aimed to determine the effects of age at training to virtual fencing technology on (1) the efficiency of associative pairing of a benign audio cue with an aversive electrical stimulus, and (2) the retention of this associative training over a long period of time without reinforcement. We predicted that virtual fencing training early in ontogeny (i.e., $\leq$ 12-months of age) would increase the rate of associative learning compared to training close to calving age, and that early training would improve the responsiveness of heifers to virtual fencing when re-trained $\geq$ 10-months later.

MATERIALS AND METHODS

Ethical Statement

All animal procedures were conducted with institutional animal ethics approval obtained prior to the start of the experiment (University of Tasmania Animal Ethics Committee A0017004).

Animals and Experimental Design

This experiment was conducted over 17 months at the Tasmanian Institute of Agriculture Dairy Research Facility (TDRF) (41°08′S, 145°77′E; 155.0 m a.m.s.l), Elliott, north-west Tasmania, Australia. Fifty-nine weaned Holstein dairy heifers (*Bos Taurus* L.) were studied from ∼6-months of age (mean ± sd; 185 ± 6.2 days). Heifers were separated from their dams at birth and housed in semi-enclosed pens (3 walls and a roof; 3.5 × 7 m) of 10 to 12 animals until weaning at ∼90 days. After weaning, heifers were housed at pasture in a single mob of 138 similarly aged females and managed as per normal commercial practice. Heifers were fed a primarily pasture-based diet that was supplemented with silage when required. Water was supplied *ad libitum*. All studied heifers were impregnated via artificial insemination at ∼15-months of age.

The experimental timeline is visually presented in **Figure 1**. The studied heifers were selected from the larger cohort of 138 animals and allocated to four treatments that differed in the age at which the naïve animals underwent training of the pairing of the audio and electrical stimuli. The treatments were as follows: **6M**—initial training at 6-months of age (n = 15), **9M**—initial training at 9-months of age (n = 15), **12M**—initial training at 12-months of age (n = 15), and **22M**—initial training at 22-months of age (n = 14). Typical of seasonal calving patterns, a period of 3-months separated the oldest and the youngest heifer in the cohort, so animals were assigned to treatments to reduce variation in age within treatments. The mean, standard variation, and coefficient of variation in the age and weights of heifers at the time of their initial training (i.e., at 6, 9, 12, or 22-months of age) are presented in **Table 1**. Heifers in the 22M

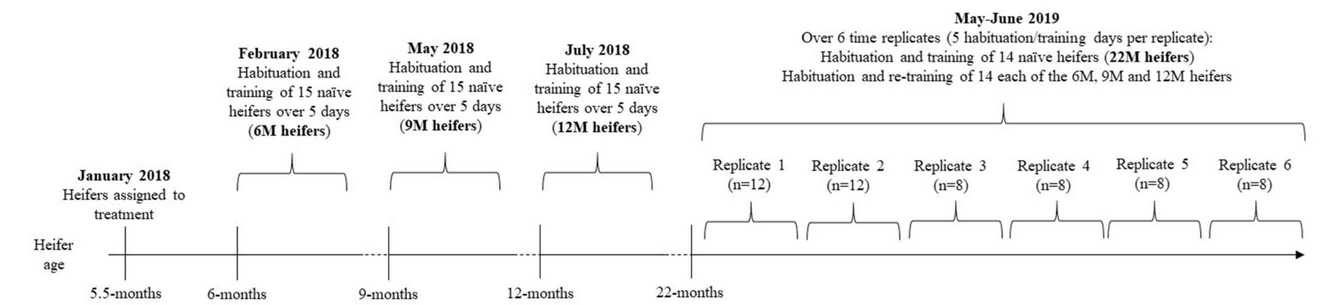

FIGURE 1 | Experimental timeline. Groups of heifers that were naïve to virtual fencing underwent training to the association between audio and electrical stimuli at 6-months (6M), 9-months (9M), 12-months (12M), or 22-months (22M) of age. The 6, 9, and 12M heifers underwent a second round of training at 22-months of age (i.e., at the same time as the naïve 22M heifers). The second round of training was conducted over 6 time-replicates, with even representation of treatments within each replicate. Treatment heifers were separated from the herd 3 days prior to commencement of habituation and training. Habituation to the test arenas and to collars was conducted over 3 days and training was conducted over 2 days (2 training sessions per day). This experimental design allowed for an assessment of the effects of age on rate of learning and the retainment of learning after an extended period without reinforcement.

TABLE 1 | Details relating to heifers when introduced to the virtual fencing technology at 6, 9, 12, or 22-months of age and when re-trained to the technology at 22-months of age.

	6-months	9-months	12-months	22-months
Rate of learning				
Number of animals	15	15	15	14
Time replication	1	1	1	6
Pasture offered per day (kg DM/heifer)[a]	3.8	4.7	5.6	11.6
Age (months, mean ± SD)	6.1 ± 0.06	9.0 ± 0.07	11.8 ± 0.04	22.4 ± 0.43
CV age (%)	0.91	0.79	0.36	1.9
Weight (kg, mean ± SD)	168 ± 12	229 ± 26	256 ± 35	470 ± 40
CV weight (%)	6.8	11.2	13.8	8.5
Month of training	February	May	July	May-June
Temperature (mean°C)	19	14.3	10.7	11.4
Rainfall (mean mm per day)	0	0.3	2.3	0.7
Windspeed (mean km/h)	8.3	10.3	11.3	8.3
Retention of learning				
Number of animals	14	14	14	
Time replication	6	6	6	
Age (months, mean ± SD)	22.1 ± 0.4	21.9 ± 0.4	22.2 ± 0.4	N/A
CV age (%)	1.9	1.8	1.9	N/A
Weight (kg, mean ± SD)	460 ± 26	479 ± 43	467 ± 55	N/A
CV weight (%)	5.6	8.9	11.7	N/A

[a] *As recommended by Moran and McLean (2001).*

SD, Standard deviation; N/A, Not applicable. The 22-months served as naïve control during retention of learning training.

treatment were 7-months pregnant during their initial training sessions. The seasonal calving pattern also meant that initial training of each treatment was conducted at different times of the year. The month of training and mean temperature, rainfall, and windspeed (during training hours) are detailed in **Table 1**. Fourteen heifers from the 6, 9, and 12M treatments underwent associative training for a second time when all the animals were 22-months of age (i.e., at the same time as heifers in the 22M treatment). All animals were 7-months pregnant during training at 22-months of age. Thus, the present experiment assessed the relationships between age at training of naïve heifers on (1) the rate of learning of the association between audio and electrical stimuli and (2) the retainment of this learning after an extended period of time without reinforcement (**Figure 1**).

Initial training sessions for the naïve heifers in the 6M, 9, and 12M treatments were conducted in a single week (i.e., time replicate). Restrictions on time and collar availability meant that associative training during the second training sessions (i.e., at 22-months of age) were conducted over 6 successive time replicates (**Figure 1**). An equal number of heifers from the 6, 9, 12, and 22M treatments were trained in each of these replicates ($n = 12$ heifers in replicates 1 and 2, $n = 8$ heifers in replicates 3–6; total $n = 56$ heifers).

Heifers were separated from the larger mob of animals 3 days prior to the commencement of their associative training sessions and housed as a single group in a paddock close to the training arena (< 100 m). During this period heifers received a fresh allocation of pasture each day and were provided with water *ad libitum* (see **Table 1** for details on pasture allocation). Animals remained in these groups for the duration of training after which they were returned to the larger mob of heifers.

Training of the Pairing of Audio and Electrical Stimuli

The Collars

The electronic collars used to remotely deliver the audio cues and electrical stimuli were the same as those used by Verdon et al. (2020). The collars were based on those used for dog training (ET300 Mini-educator, E-Collar Technologies, Garrett, IN, USA), fitted into a custom casing (MooMonitor$^+$, Dairymaster Inc., Kerney, Ireland) and enabled an operator to manually deliver audio or electrical stimuli through a remote control device. The range of the collar and remote-control device communication system was 800 m. The electronic collar was secured around the neck of the heifers and two electrodes that delivered the electrical stimulus were positioned to contact the skin in a shaved area behind the poll. The audio stimulus was a constant polyphonic tone (84 dB) delivered from two speakers just behind the ears of the animal. The electrical stimulus intensity was set to 3 V (120 mW), which equated to a setting of 50 on the 100-point scale provided with the remote-control device.

The Test Arena

The layout of the test arena where animals were trained to the association between the audio and electrical stimuli differed between the initial training of 6M heifers and all other training sessions. The layout of the test arena used in the initial associative training of the 6M heifers is presented in **Supplementary Figure 1** and the test arena used in all other associative training sessions is presented in this manuscript as **Figure 2**. Changes to the layout were made to improve training procedures following observations during 6M training sessions. These changes are detailed below.

Both test arenas consisted of stockyards, two temporary holding paddocks, and three training paddocks. A trough of grain was positioned at the end of the training paddock to motivate the animals to move down the far end of the paddock. To account for seasonal variability in pasture growth and quality, and to further encourage heifers to move to the far end of the paddock, pasture was mown so that only the final 10% of the paddock area contained fresh pasture. The first test arena used for initial training of 6M heifers was constructed using temporary electrified poly-wire fencing, did not include a walkway leading up to test paddock 1, and the post-test holding paddock was located 80 m beyond the end of the training paddocks (**Supplementary Figure 1**). Observations made during the 6M training sessions suggested that one or a combination of these factors were motivating heifers to challenge the virtual fence despite effective associative learning, particularly when training was being conducted in arena 1. The second test arena was built using non-electrified permanent fencing and included a 25-meter walkway between the pre-test pen and the training paddocks. The new arena also positioned the post-test holding pen in front of training paddock. This second test area is the same as that described by Verdon et al. (2020) and was utilized for all training in the present experiment excluding the initial training of the 6M heifers.

During training, animals were relocated from their paddock to the stockyards at ∼0900 h for the fitting of the electronic collars and individualized marking of both flanks using stockmarker. Heifers were then held as a single group in the pre-test paddock located in front of the training paddocks. After each habituation or training session, heifers were moved to the post-test holding paddock where they remained until all animals had been tested (habituation and training procedures are described in the following sections). Animals were able to graze available pasture in the pre- and post-test holding paddocks (<1800 kg DM/ha) and water was provided *ad libitum*. Collars remained fitted for the two habituation or training sessions held each day (session 1 between 10:00 and 11:00, session 2 between 14:30 and 15:30). At ∼16:00, heifers were moved back to the stockyards where collars were removed before animals were returned to their paddock.

Habituation Procedures

Heifers underwent a 3-day habituation period prior to training to familiarize them with the test areas and the location of the feed attractant. The electronic collars were not activated during the six habituation sessions (2 per day). Heifers were introduced to the training paddocks in groups of 5 for the first habituation, in pairs for the second and individually for habituation sessions 3–6. The training paddock being utilized was rotated with each session, ensuring that heifers received two habituations per paddock (one AM and one PM). Heifers were given free access to the training paddock during habituation and provided with as much time as required to start consuming the grain. Once feeding commenced, heifers were permitted to feed for 3 min. By the final habituation day, all animals began consuming grain following entry to the training paddock in a median of 40 s (range 14–140 s) during the initial training of naïve animals and 26.5 s (range 9.5–139 s) during the retention of learning training sessions at 22-months of age.

Training Procedures

Four sessions of training of the pairing of the audio and electrical stimuli with activated collars were held over 2 days immediately following the habituation period. This number of sessions was chosen because previous research found that from the fourth training sessions heifers could be categorized as consistently avoiding interacting with the virtual fence or consistently tolerating the electrical stimuli to reach the feed attractant (Verdon et al., 2020). Individual heifers were introduced to the test arenas during each training session. The training paddock

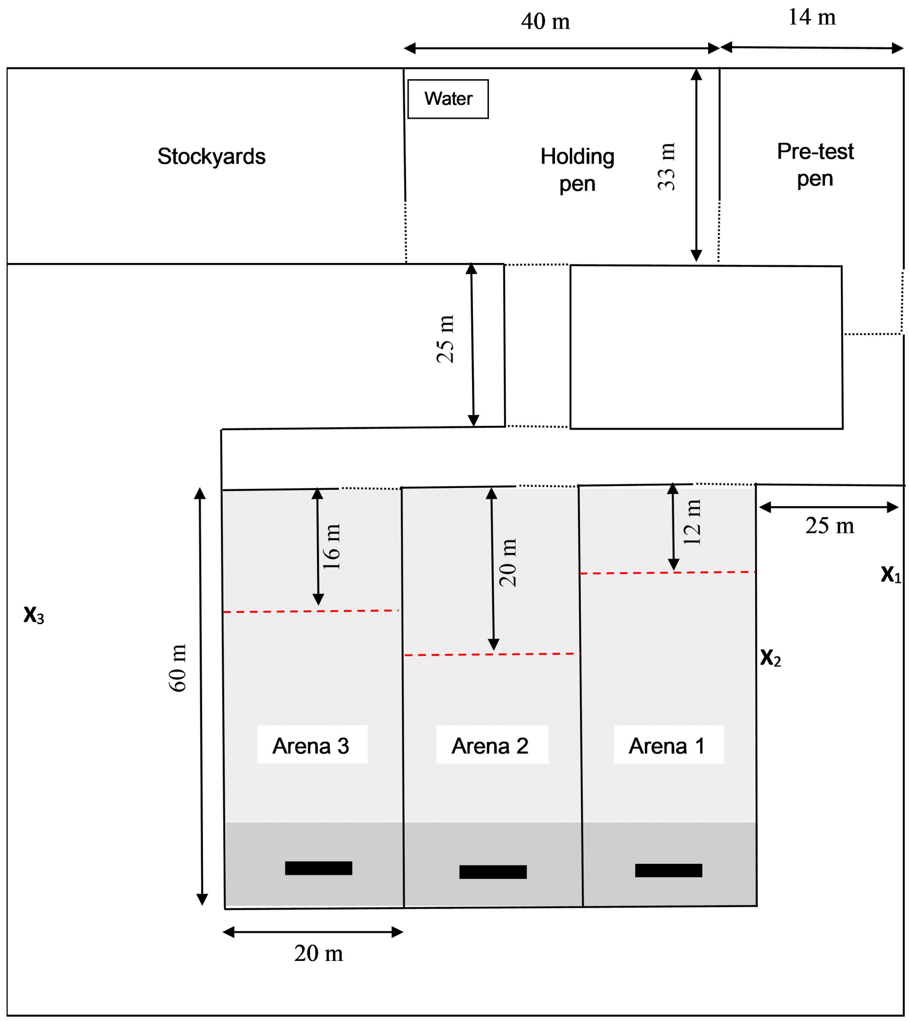

FIGURE 2 | Layout of the test arena where training of the association between audio and electrical stimuli was conducted. The test arena consisted of stockyards, pre- and post-test holding pens and three training arenas which were rotated between training sessions. Collars that delivered the audio and electrical stimuli to heifers were fitted in the stockyards after which animals were housed as a group in the pre-test pen. Individual heifers were removed from the pre-test pen for associative training and housed in the holding pen after training. A trough of grain was positioned at the far end of the training paddock (indicated by black rectangles, ▬) to motivate animals to move down the paddock. Pasture was mown for the first 90% of each arena (□) leaving longer pasture as an additional attractant at the far end (■). During training, a virtual fence boundary separated an "inclusion zone" (i.e., area in which animals could move freely) from an "exclusion zone" (i.e., area beyond which stimuli would be applied). The exclusion zone was set at a different length from the entry of each test arena, as indicated by red dashed line (---). The position of video cameras recording heifer behavior and of the researcher responsible for manually administering the audio and electrical stimuli via a remote control device are indicated by X_1, X_2, and X_3 for training arenas 1, 2, and 3 respectively. Lockable gates are represented by a dotted line (…). Note that the test arena differed slightly during the introduction training for 6-month old heifers (see **Supplementary Figure 1** for this test arena).

being utilized followed the same rotation as that used during habituation. A virtual fence boundary separating an "inclusion zone" (i.e., area in which animals could move freely) from an "exclusion zone" (i.e., an area beyond which the audio and electrical stimuli would be applied) was established at either 12, 16, or 20 m from the entrance to the training paddock, depending on the paddock being utilized. Distances varied between paddocks to delay animals learning an association between delivery of stimuli and the location of the exclusion zone. A researcher with experience in using the manual collars for training heifers was located ∼20 m outside the training paddock to administer audio or electrical stimuli remotely (**Figure 2**). There was no visual indication of the exclusion zone apart from a small amount of white tape on the fence to aid the researcher.

The following procedures determined the application of the audio and electrical stimuli by the researcher, as utilized by Verdon et al. (2020). Based on the researcher's visual estimation, the audio stimulus was applied for 2 s as the heifer entered the exclusion zone. If the heifer stopped moving further into the exclusion zone, the application of the audio stimulus immediately ceased. If the heifer continued to move forward, however, an electrical stimulus (< 0.5 s) was immediately administered by the researcher. If the heifer recommenced or continued movement into the exclusion zone after the delivery of the electrical stimulus, the audio stimulus was re-applied, immediately followed by another electrical stimulus if again she continued to move into the exclusion zone. No further stimuli were applied to an animal unless she was further proceeding into the exclusion zone. The training session concluded if (1) no breaches into the exclusion zone were made within 3-min of entry into the training paddock, (2) a period exceeding 3-min separated two breaches into the exclusion zone, or (3) a maximum number of five electrical stimuli were delivered. Heifers were videotaped during training (Panasonic camcorder, model NV-DS60; Panasonic Corporation, Osaka, Japan) so that their behavior could be translated at a later date.

Measures Recorded

The following data were collected during both the initial training sessions of naïve heifers and the retention of learning training sessions conducted at 22-months of age. The number of interactions with the virtual fence and the number of audio and electrical stimuli delivered were recorded *in situ* and confirmed by a single observer that was blinded to treatment using video records. The number of interactions the heifer had with the virtual fence before responding to the audio cue alone was retrospectively determined using these stimulus data. The proportion of interactions with an electrical stimulus was calculated as the number of electrical stimuli delivered ÷ the number of audio cues delivered. The following measures were also obtained from video records taken during the initial training sessions of naïve animals by a single observer: the time taken to interact with the virtual fence; the time taken for the heifer to reach feed attractant; the behavioral response of heifers to the audio or electrical stimuli (see **Table 2** for ethogram). The ethogram for behavior observations was adapted from Verdon et al. (2020) but to improve inter-observer reliability the behavioral response of animals immediately following the application of the stimulus was assessed (i.e., within 2 s). Observations on a subset of 7 heifers were repeated by the original observer and by one other observer. This determined high intra-observer reliability for most behaviors ($r^s \geq 0.95$) and high inter-observer reliability for all behaviors ($r^s \geq 0.94$). A single discrepancy lowered the intra-observer reliability for the behavior "stop feeding/grazing" ($r^s = 0.76$), but this is still considered an acceptable level of agreement (Martin and Bateson, 1993).

TABLE 2 | Ethogram of cattle behaviors recorded during associative training. Intra-observer reliability $r^s > 0.76$, inter-observer reliability $r^s > 0.94$.

Walk forward	Moving forward one leg at a time with an even gait. Movement continues for more than one body length
Run forward	Moving forward at a pace that is faster than a walk. The head is typically held up. Movement continues for more than one body length
Buck and run forward	Both hind legs off the ground and extended backwards in combination with run forward. Several bucks may be observed as the heifer moves forward
Continue feeding/grazing	Heifer is feeding from the trough or grazing at the time of stimulus delivery, and continues the feeding behavior without lifting her head (i.e., above height of brisket). Heifer may flinch or momentarily pause her chewing
Stop	Within one body length following stimulus delivery, heifer stops moving with all four feet on the ground and is stationary for at least 2 s
Stop and graze	Within one body length following stimulus delivery, heifer stops moving forward and commences grazing. Heifer may be stationary when grazing, or may turn to the side or turn back while grazing
Stop feeding/grazing	Heifer is feeding from the trough or grazing and lifts head (i.e., above height of the brisket) following stimulus delivery. Heifer may turn or step away and/or continue to chew.
Turn back	Full body turn of 135–215° so heifer is facing toward the inclusion zone. Heifer may remain stationary, or may walk/run back toward the inclusion zone
Turn to the side	Full body turn of 45–135° so heifer is parallel (or almost parallel) to the virtual boundary, heifer may remain stationary or move parallel to the boundary

Statistical Analysis

Analyses were restricted to use of data collected from the first three training sessions. This decision was made on the basis that only around 50% of animals interacted with the virtual fence at the fourth training session during both the initial training of naïve animals and the retention of learning training at 22-months of age (Cochran's Q-test—Initial training $\chi^2(3) = 24.1$, $P < 0.001$; Retention of learning training $\chi^2(3) = 39.5$, $P < 0.001$; **Figure 3**). Similar findings were reported by Verdon et al. (2020). As such, only data from sessions 1–3 were considered most representative of the associative learning process. Data relating to the time taken to interact with the virtual fence and for the heifer to reach the feed attractant were averaged over training sessions 1 to 3. The number of audio cues and electrical stimuli delivered were summed over sessions 1–3. The proportion of total interactions with the virtual fence in which an electrical stimulus was delivered was calculated from the summed stimuli data. Behavioral responses of individual heifers to the audio cue or electrical stimuli during the initial training sessions were calculated as a proportion of total behavioral responses (to audio or electrical stimuli) observed over sessions 1–3.

All statistical analyses were carried out using the SPSS statistical software package (SPSS 26.0, SPSS Inc., Chicago, IL, USA) and the unit of analyses was the individual heifer. The effects of age at training on the rate of learning (initial training at 6, 9, 12, or 22M) and retention of learning (all animals trained at 22-months of age) were analyzed separately using generalized linear mixed models (GLMM). A visual inspection of the quantile-quantile plots and histograms were conducted prior to both sets of analysis. Data relating to the proportion of interactions in which an electrical stimulus was delivered were subsequently arcsine square-root transformed while duration data were logarithmically transformed (time to interact with

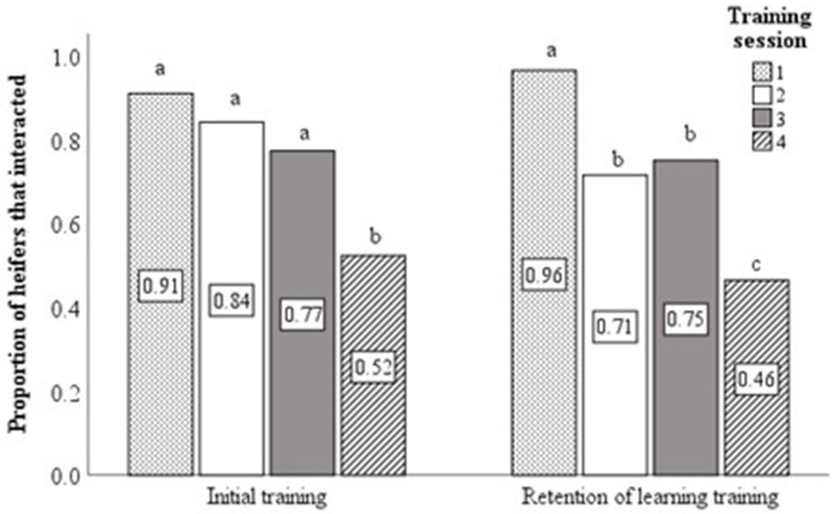

FIGURE 3 | Using data from the four training sessions, bar charts displaying the proportion of heifers that interacted with virtual fence during the initial ($n = 59$) and retention of learning ($n = 56$) training sessions. Different superscript letters[abc] show where treatment means differ.

the virtual fence, time to reach feed attractant), so that residual variation was homogenous between treatments.

Effects of Age on Rate of Learning

Differences in the design of the test arena meant that the 6M treatment could not be included in the analysis on the effects of age at training on the rate of associative learning. A technical malfunction at day 1 (training sessions 1 and 2) also meant that the time 6M heifers took to interact with the virtual fence and their behavioral response to audio and electrical stimuli could not be obtained from video records. For the 6M heifers, the proportion of interactions with an electrical stimulus, the total number of interactions with the virtual fence and the time to reach the feed attractant are presented to facilitate a descriptive comparison to the other treatments.

The initial training sessions of naïve animals were conducted in a single time replicate for 6, 9, and 12M treatments, but 22M heifers were trained over 6 successive time replicates. A new factor named "time replicated" was created to account for this unbalanced replication. The treatments that were not replicated in time (i.e., 9 and 12M) were assigned to one level of this factor, while the 22M treatment that was replicated over time was assigned to a second level. The effect of treatment (i.e., initial training at 9, 12, or 22M) nested within "time replicated" was then included in each analysis as a fixed factor. The number of interactions the heifer had with the virtual fence before responding to the audio cue alone and the total number of audio cues delivered were analyzed with a poisson distribution and log link. The time heifers took to interact with the virtual fence, the proportion of interactions that included an electrical stimulus, and the time taken for them to reach the feed attractant were analyzed with a normal distribution and identify link. Heifers that did not breach the exclusion zone were recorded as missing values for the time to interact with the virtual fence or reach feed attractant. As such, the Satterwaite approximation was used to calculate degrees of freedom. The behavioral response of heifers to the audio and electrical stimuli were analyzed for treatment effects using a non-parametric Kruskal-Wallis test. A separate analysis was conducted for each behavior observed after administration of the audio cue or the electrical stimulus. Heifers that did not breach the exclusion zone were recorded as missing values in these analyses. Inter- and intra-observer reliabilities for each behavior were assessed using non-parametric spearman rank correlations.

Effects of Age on Retention of Learning

All heifers were 22-months of age during the retention of learning training sessions. Each model included the main effect of treatment (heifers that were initially trained at 6, 9, 12M and undergoing a second round of training compared to naïve 22M heifers undergoing their first round of training) and time replicate (1–6) as a random blocking factor. The number of interactions the heifer had with the virtual fence before responding to the audio cue alone was analyzed with a poisson distribution and log link, while the proportion of interactions that included an electrical stimulus was analyzed with a normal distribution and identify link. A Wilcoxon Signed Rank test was used to examine differences in the proportion of interactions with the virtual fence that included an electrical stimulus during the initial and second training sessions for 6, 9, and 12M treatments.

RESULTS

To aid with interpretation, raw means are presented with estimated marginal means ± SEM (and backtransformed estimated marginal means where relevant) presented in **Supplementary Table 1**.

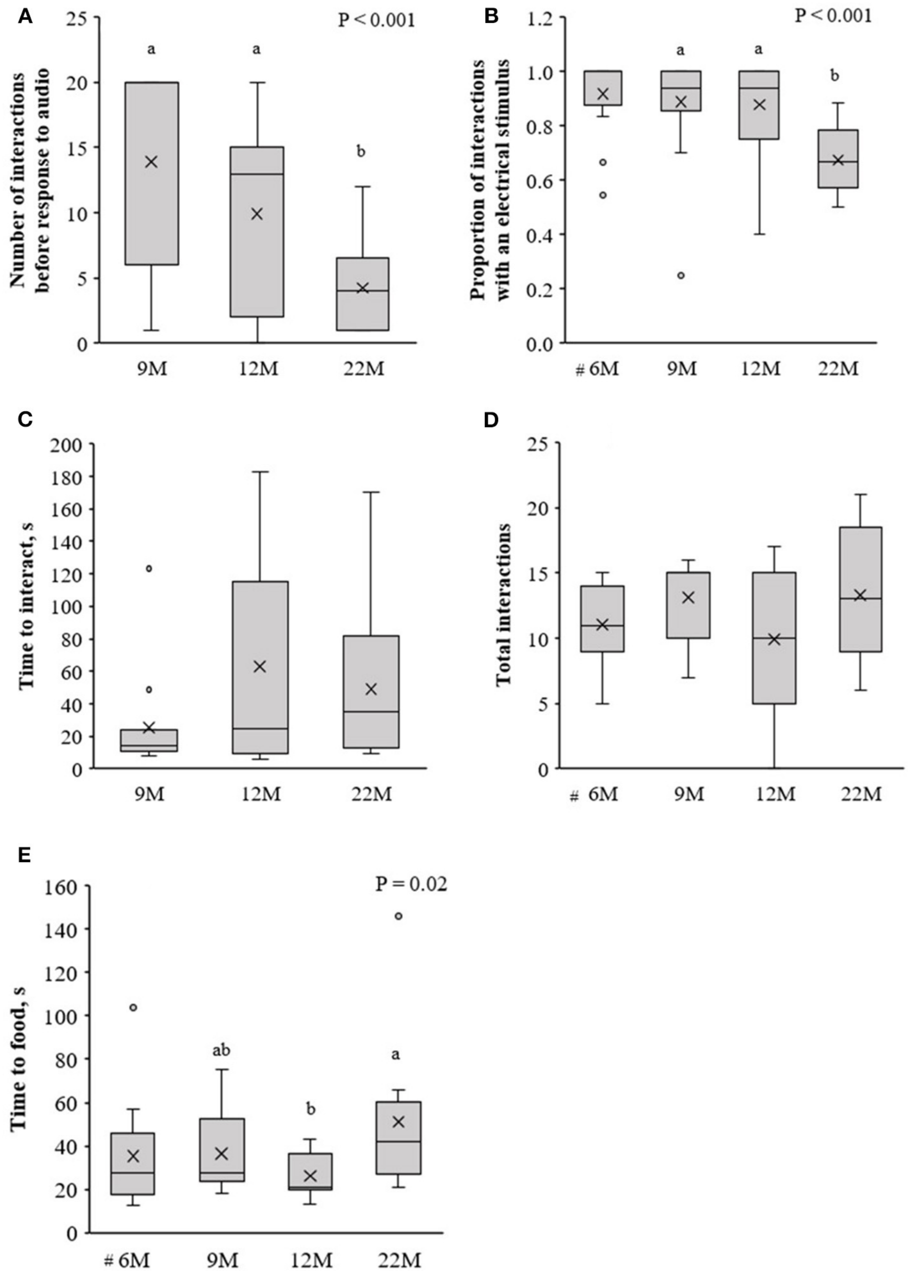

FIGURE 4 | Naïve heifers that were trained to the virtual fencing technology at 6, 9, 12, or 22-months of age (6, 9, 12, 22M, respectively). Using data from the first three training sessions of naïve animals, **(A)** the number of interactions with the virtual fence before the heifer responded to the audio cue alone, **(B)** the proportion of interactions with the virtual fence during which an electrical stimulus was delivered, **(C)** the time to interact with the virtual fence, **(D)** the total number of interactions with the virtual fence, and **(E)** the time to reach the feed attractant. #The test arena layout differed for the initial training of 6M heifers compared to all other training sessions, so these animals were not included in this analysis. Raw data are presented. Estimated marginal means are presented in **Supplementary Table 1**. Boxplots show the median and the first and third quartiles (25 and 75% of data), with whiskers extending to the lowest and highest values. Values >1.5 × the interquartile range (IQR) are indicated by o. Different superscript letters[abc] show where treatment means differ.

Effects of Age on Rate of Learning

Age at training affected the rate of learning of the association between audio and electrical stimuli. Heifers that were trained at 22-months of age (called 22M) required fewer interactions with the virtual fence before responding to the audio cue alone, compared to training at an earlier age [i.e., 9 or 12-months; called 9 and 12M, respectively; $F_{(2, 40)} = 11.4$, $P < 0.001$; **Figure 4A**]. Consequently, the proportion of interactions with the virtual fence in which an electrical stimulus was delivered was lower at 22M than 9 or 12M [$F_{(2, 40)} = 21.2$, $P < 0.001$; **Figure 4B**]. Data

TABLE 3 | The effect of age at training of the pairing of audio and electrical stimuli (9, 12, or 22-months of age) on the behavioral response to audio and electrical stimuli ($n = 42$).

Behavioral response[1]	9-months		12-months		22-months		χ^2 (2)	P-value
	Median	Range	Median	Range	Median	Range		
Audio stimulus								
Buck and run forward	0.00	0.00–0.25	0.00	0.00–0.17	0.00	0.00–0.29	0.33	0.85
Run forward	0.40[a]	0.00–0.60	0.67[a]	0.00–0.80	0.20[b]	0.00–0.53	10.5	0.005
Walk forward	0.13	0.00–0.93	0.22	0.00–0.75	0.32	0.00–0.68	1.18	0.55
Continue grazing	0.31	0.00–0.53	0.07	0.00–0.33	0.19	0.00–0.35	5.64	0.06
Stop	0.00[a]	0.00–0.08	0.00[a]	0.00–0.08	0.03[b]	0.00–0.40	3.31	0.01
Stop and graze	0.00	0.00–0.00	0.00	0.00–0.08	0.00	0.00–0.14	5.02	0.08
Stop feeding/grazing	0.00[a]	0.00–0.00	0.00[a]	0.00–0.00	0.00[b]	0.00–0.17	11.0	0.004
Turn back	0.00[a]	0.00–0.38	0.00[a]	0.00–0.25	0.08[b]	0.00–0.20	9.78	0.008
Turn to the side	0.00	0.00–0.20	0.00	0.00–0.11	0.08	0.00–0.21	3.62	0.16
Electrical stimulus								
Buck and run forward	0.00	0.00–0.43	0.20	0.00–0.44	0.00	0.00–1.00	1.40	0.50
Run forward	0.29	0.00–0.53	0.33	0.00–0.80	0.18	0.00–0.75	4.49	0.11
Walk forward	0.07	0.00–0.87	0.14	0.00–0.29	0.15	0.00–0.73	1.50	0.47
Continue grazing	0.22[a]	0.00–0.53	0.00[b]	0.00–0.50	0.05[b]	0.00–0.25	9.70	0.008
Stop	0.00	0.00–0.25	0.00	0.00–0.07	0.00	0.00–0.20	0.40	0.82
Stop and graze	0.00	0.00–0.00	0.00	0.00–0.14	0.00	0.00–0.17	3.47	0.18
Stop feeding or grazing	0.00	0.00–0.33	0.00	0.00–0.33	0.15	0.00–0.50	2.72	0.26
Turn back	0.00	0.00–0.50	0.00	0.00–0.67	0.00	0.00–0.20	0.67	0.71
Turn to the side	0.00	0.00–0.29	0.00	0.00–0.17	0.00	0.00–0.10	1.03	0.60

[1]*As a proportion of all behavioral responses totaled over three training sessions. Different superscript letters[a,b] show where treatments differ.*

from the heifers trained at 6-months of age (called 6M) were not included in these analyses, but a descriptive comparison suggests 6M heifers behaved more similarly to 9 and 12M heifers than the 22M heifers (**Figures 4A,B**). Age at training did not affect the time it took heifers to interact with the virtual fence [$F_{(2, 39)} = 2.5$, $P = 0.1$; **Figure 4C**] or the total number of interactions [$F_{(2, 41)} = 1.5$, $P = 0.23$; **Figure 4D**], but heifers trained at 12M reached the feed attractant more quickly than 22M heifers [$F_{(2, 29)} = 4.5$, $P = 0.02$; **Figure 4E**].

Behavior During Training

Nine different behavioral responses were observed following both the delivery of the audio cue and electrical stimuli (**Table 3**). The most frequently observed responses to the audio cue included run forward (34% of responses), walk forward (28% of responses), or continue grazing (19% of responses). The most frequently observed responses to the electrical stimuli were run forward (30% of responses), walk forward (18% of responses), continue grazing (14% of responses), and buck while running forward (13% of responses). Compared to the 22M heifers, following the audio cue, animals in the 9 and 12M treatments were more likely to run forward and less likely to stop (moving forward or feeding) and turn back (**Table 3**). Heifers in the 9M treatment were more likely to continue grazing following an electrical stimulus than 12 and 22M heifers (**Table 3**). There were few other effects of age at training on the behavioral response of heifers to electrical stimulus (see **Table 3**).

Effects of Age on Retention of Learning

There were no beneficial effects of previous training at an early age (i.e., 6, 9, or 12-months of age) on the responsiveness of heifers to the audio and electrical stimulus when re-trained at 22-months of age. Compared to naïve 22M heifers, animals that were initially trained at 6 and 9M required more interactions with the virtual fence before responding to the audio cue alone, with 9M heifers requiring the highest number of interactions [$F_{(3, 52)} = 10.5$, $P < 0.001$; **Figure 5A**]. There was an effect of treatment on the proportion of interactions in which an electrical stimulus was delivered [$F_{(3, 58)} = 2.8$, $P = 0.046$; **Figure 5B**]. The 9M heifers received a higher proportion of electrical stimuli compared to 12M heifers and tended receive a higher proportion than 22M heifers (LSD test $P = 0.055$). Heifers that underwent training at 6 and 12M did not differ from the naïve heifers in the proportion of interactions with an electrical stimulus.

Wilcoxon Signed Rank tests showed that the proportion of interactions with an electrical stimulus was higher during initial training than during re-training for the 6M ($Z = -2.5$, $P = 0.013$), 9M ($Z = -2.1$, $P = 0.04$), and 12M ($Z = -3.0$, $P = 0.003$) treatments. The reduction in the proportion of interactions with electrical stimulus over time was more pronounced for 6 and 12M heifers than for 9M heifers.

DISCUSSION

This experiment used a manual training collar to assess the effect of dairy heifer age at training on the rate of learning

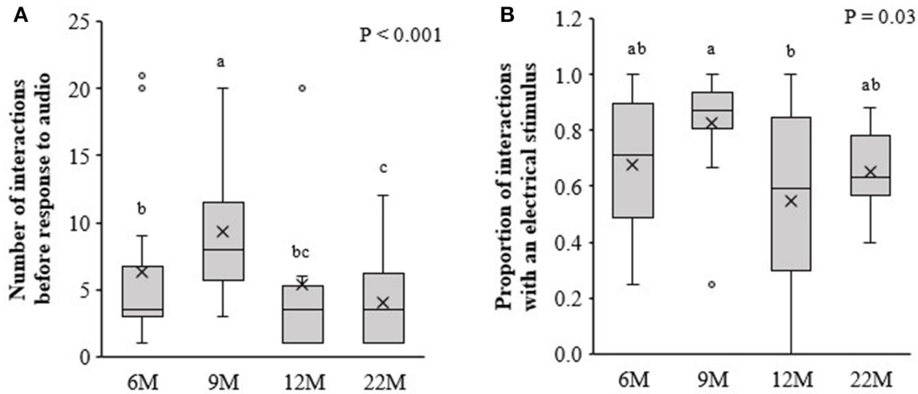

FIGURE 5 | Retention of learning: Heifers that were introduced to virtual fencing at 6, 9, or, 12-months of age (6, 9, and 12M, respectively) and re-trained at 22-months of age, alongside a cohort of naïve heifers being introduced to virtual fencing at 22-months of age (22M). Using data from the first three retention of learning training sessions, **(A)** the number of interactions with the virtual fence before the heifer responded to the audio cue alone and **(B)** the proportion of interactions with the virtual fence during which an electrical stimulus was delivered. Raw data are presented. Estimated marginal means are presented in **Supplementary Table 1**. Boxplots show the median and the first and third quartiles (25 and 75% of data), with whiskers extending to the lowest and highest values. Values >1.5 × the interquartile range (IQR) are indicated by o. Different superscript letters[abc] show where treatment means differ.

of the association between a benign audio cue and aversive electrical stimulus, and on the retention of this learning over a long period without reinforcement. Contrary to expectations, the rate of learning was accelerated in older (i.e., 22-months of age) compared to younger heifers (i.e., 6, 9, or 12-months of age), and there were no beneficial effects of previous training at an early age on the responsiveness of heifers when re-trained ≥10-months later.

Effects of Age on Rate of Learning

Heifers that underwent training at 22-months of age took fewer interactions to respond to the audio cue alone, received a lower proportion of electrical stimuli, and were less likely to run forward and more likely to stop moving forward after the application of the audio cue, compared to the younger heifers. Reproductive status is one important difference between the older and younger heifers in this experiment, as the older heifers were ∼7-months pregnant at training. Humans and animals experience significant hormonal and neurochemical changes during pregnancy (reviewed by Kim, 2016). These changes may affect the salience of the stimuli delivered during training. For example, pregnant women show heightened neural activity and increased attention to pictures of fearful or angry faces, particularly when tested toward late pregnancy (Kim, 2016). This maternal hyper-vigilance is thought to maximize the protection of offspring from potential dangers (Barba-Müller et al., 2019). Thus, hormonal changes related to pregnancy may have increased the attention of the 22-month old heifers to the electrical stimulus, allowing them more quickly to learn to control receipt of the stimulus through behavioral change.

A second explanation for the faster rate of learning observed in the 22-month old heifers is that their older age had provided greater opportunities for experiences that improve associative learning or other cognitive skills. For example, Verdon et al. (2020) found that experience with electric fencing resulted in more rapid associative pairing of audio and electrical stimuli in dairy heifers compared to those that had no experience with electric fencing, and the more interactions a heifer had with an electric fence the more responsive she was to the audio cue during associative training. Compared to their younger counterparts, the older heifers in this experiment would also have had greater exposure to the unpredictable environmental changes common in pasture-based dairy systems (e.g., variable environmental conditions, movement to new areas of the farm, variability in the quantity, quality and composition of feed, interactions with humans). Exposure to environmental unpredictability may strengthen cognitive skills by inducing higher levels of neuronal plasticity allowing the animal to cope better with fluctuating environmental conditions (Taborsky, 2017). This has been demonstrated by research in fish, where environmental uncertainty induced higher levels of neuronal and behavioral plasticity as well as improved cognitive abilities (Kotrschal and Taborsky, 2010; Roy and Bhat, 2016; Carbia and Brown, 2019).

The motivation of younger heifers to access the feed attractant may have been greater than for the older heifers, making them more willing to tolerate the electrical stimulus. According to the framework proposed by Mendl and Paul (2020), a heifer's current internal status (e.g., state of hunger) would affect its estimation of the value, or rewarding properties, of the feed attractant. A feed attractant trial with a similar design to the present experiment observed the proportion of electrical stimuli delivered to 6-month old heifers to decline after the provision of fresh silage (Verdon et al., 2020). We consider this likelihood to be low in the present experiment, however, because all animals were fed to recommendations for their age. The lack of time replication in the training of younger heifers (training conducted over 1 week compared to over 6 weeks for the 22-month heifers) is a limitation of this research that prevents us from accounting for any variation in the rate of learning within treatments that could be associated with fluctuations in feeding (e.g., quality of pasture) or environmental conditions.

Effects of Age on Retention of Learning

Relative to naïve 22-month old heifers, animals that were initially trained at 9-months required more interactions with the virtual fence before responding to the audio cue alone and tended to have more interactions with an electrical stimuli when re-trained at 22-month old, however, there were few differences between heifers that underwent training at 6 or 12-months of age and the naïve heifers. These findings suggest that heifers did not retain their associative learnings over a long period (i.e., ≥ 10-months). Other research on adult cows shows that they can be trained by conditioning to a stimulus using negative reinforcement, but that behavior is soon extinguished without regular stimulus use (Albright, 1981). Our data indicate that stock need to be re-trained following an extended period of time without virtual fencing technology (e.g., heifers that have been reared by a contractor, dry cows that have been moved to a different location during periods of low pasture growth), however, more research is required to determine how long the audio and electrical stimuli association is maintained.

The 9-month old Holstein heifers in this experiment would likely have been in the early adolescent period of development at the time of their initial training, given that Holstein-Friesian heifers experience their first ovulation at an average of 9.5-months (Wathes et al., 2014). Adolescence is characterized by dramatic neurophysiological, hormonal and behavioral change (Lo Iacono and Carola, 2018). A recent study suggests that individual consistency in the behavioral response of dairy cattle to novelty is poor across the developmental period of puberty compared to pre-weaning and in the first lactation (Neave et al., 2020). The HPA-axis and brain regions involved in learning, memory and higher cognitive abilities (e.g., behavioral suppression, attention and decision making) are highly plastic and particularly susceptible to environmental stressors during adolescence (see reviews by Green and McCormick, 2013; Baker et al., 2014; Lo Iacono and Carola, 2018). We thus hypothesize that the stressful experience of associative training during the sensitive early adolescence developmental period caused neurological changes with possible long-term effects on emotionality and/or cognitive processing for the 9-month old heifers in this experiment.

Scientific understanding of the physiological and behavioral effects of stress during adolescence has been developed primarily through research on rodents under laboratory conditions. Morrissey et al. (2011) exposed adolescent or adult rats to 16 days of social instability stress, followed by fear conditioning weeks later. The adolescent and adult rats did not differ in rate of conditioning but stress during adolescence decreased context and cue memory later in life. Other evidence from laboratory rats shows that stress during adolescence increases fear or anxiety like behavior (e.g., Brydges et al., 2012; Yee et al., 2012; Müller et al., 2018) and risk-taking in the adult (Toledo and Sandi, 2011; Brydges et al., 2012; Traslaviña et al., 2014). We are unable to conclude whether the reduced performance of the 9-month old heifers during re-training in this experiment is related to impaired cognitive function (e.g., behavioral suppression, attention, or decision making) or changes in emotional state (e.g., anxiety). Parts of the human brain relating to stimulus salience, which is used in threat detection, and emotional regulation, used in behavioral suppression, are frequently co-activated (Barba-Müller et al., 2019). Indeed, a major function of emotional states is to organize and guide behavioral choices (Mendl and Paul, 2020). Thus, dysfunction in the cognitive domain is often related to dysfunction in the emotional domain and vice versa (see review by Green and McCormick, 2013).

An emotionally resilient cow with high cognitive abilities may be better equipped to cope with the increasingly complex environment provided by modern dairy farms (in terms of uptake of technologies and herd sizes). This includes the possible use of virtual fencing to implement increasingly intensive and complex grazing regimes in pastoral dairy systems (e.g., Verdon et al., 2018). We encourage future work to assess the effects of stress at sensitive periods of ontogeny on the development of emotionality and cognition in farm animals.

CONCLUSIONS

Results of this experiment indicate that training heifers close to calving age (i.e., 22-months) achieves more rapid associative pairing of audio and electrical stimuli compared to training at a younger age (≤ 12-months). There were no benefits of previous training at an early age on the responsiveness of heifers when re-trained at 22-months of age, and some evidence that the experience of associative training during early adolescence (at about 9-months of age) has long-term negative effects on emotionality and/or cognitive processing. We conclude that dairy heifers should be trained to virtual fencing technology close to calving age rather than earlier in their ontogeny and recommend that stock be re-trained following an extended period without virtual fencing technology.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by University of Tasmania Animal Ethics Committee A0017004. Written informed consent for participation was not obtained from the owners because these animals were University owned livestock for research purposes.

AUTHOR CONTRIBUTIONS

MV and RR contributed conception and design of the experiment. MV conducted the experiment, organized the database, performed the statistical analyses, and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2020.618070/full#supplementary-material

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3-Nitrooxypropanol Decreased Enteric Methane Production From Growing Beef Cattle in a Commercial Feedlot: Implications for Sustainable Beef Cattle Production

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Effects of the investigational methane (CH_4) inhibitor 3-nitrooxypropanol (3-NOP) on animal performance, health and enteric CH_4 production of beef cattle were evaluated in a commercial feedlot. Two concurrent studies were conducted: a large pen study (4,048 cattle, eight pen replicates per experimental group) to measure animal performance and health and a small pen study (a subset of 50 cattle from the large pen study, $n = 25$ per experimental group) to measure enteric CH_4 emissions. Within the study, animals (body weight ± SD, 282 ± 8 kg) were assigned in a completely randomized design to one of two groups: control, fed a backgrounding diet (70% corn or barley silage, 30% steam-flaked barley grain concentrate; dry matter (DM) basis) and 3-NOP, fed the backgrounding diet containing 3-NOP. The treatment group in the large pen study was adapted to 3-NOP (12 ± 3 d) before receiving the final target level of 200 mg/kg of DM, which was fed for 108 ± 8 d. Animals in the small pen CH_4 emissions study received a basal diet or a basal diet with 3-NOP, with the dose increased every 28 d: low (150 mg/kg DM; 1.27 g/d), medium (175 mg/kg DM; 2.25 g/d), and high (200 mg/kg DM; 2.75 g/d). Intake in the small pens was monitored by electronic feeding bunks and CH_4 was measured using the GreenFeed system. In the large pen study, total weight gained, average daily gain, and animal health variables were not affected by 3-NOP, but DM intake (DMI) tended to decrease ($P = 0.06$) by 2.6% relative to control (8.07 kg/d), while gain:feed ratio tended to be improved ($P = 0.06$) by 2.5% relative to control (0.161 kg weight gain/kg DMI). In the small pen study, average daily consumption of 3-NOP increased with inclusion rate whereas average DMI was decreased by 5.4% ($P = 0.02$) compared with control (10.4 kg/d). On average, addition of 3-NOP decreased ($P = 0.001$) CH_4 emissions (g/d) by 25.7% and yield (g CH_4/kg DMI) by 21.7%. In conclusion, supplementing a backgrounding diet with 3-NOP decreased CH_4 yield and tended to improve feed efficiency of beef cattle fed in a commercial feedlot with no negative impacts on animal health.

Keywords: beef cattle, enteric methane, environmental sustainability, greenhouse gas emissions, methane inhibitor

INTRODUCTION

As countries move toward greenhouse gas (GHG) emission neutrality by 2050 there is increasing pressure on ruminant livestock production to reduce enteric methane (CH_4) emissions. The Special Report of the International Panel on Climate Change [Intergovernmental Panel on Climate Change (IPCC), 2018] calls for a 24–27% reduction in CH_4 emissions from agriculture in order to limit a potential temperature increase to 1.5°C. Methane has a much shorter lifetime (half-life; 8.6 years) than carbon dioxide (CO_2) in the atmosphere (>100 years, Muller and Muller, 2017), which makes it attractive for short-term gains in global warming abatement. Enteric CH_4 from ruminants comprises ∼4–6% of global anthropogenic GHG emissions (40% of all livestock emissions; Gerber et al., 2013). As a result, there is an urgent need to develop technologies and mitigation strategies that can be cost-effectively adopted by cattle producers to lessen the contribution of ruminant livestock to GHG emissions (Beauchemin et al., 2020).

Ruminant livestock produce enteric CH_4 as an end product of feed digestion. In the rumen, polysaccharides (mainly cellulose, hemicellulose, and starch) are hydrolyzed to glucose and other hexoses and pentoses, with the monosaccharides further metabolized to volatile fatty acids, CO_2 and dihydrogen (H_2). The volatile fatty acids are used by the animal as a main source of energy, while CO_2 and H_2 are used to form CH_4, which is eructated to the atmosphere via the breath. This process allows ruminants to derive nutrients from forages and other cellulosic materials, thereby avoiding direct competition with humans. However, CH_4 is a potent GHG with a global warming potential of 28-times that of CO_2 [over a 100-year period, Intergovernmental Panel on Climate Change (IPCC), 2013].

A broad range of potential mitigation strategies has been proposed to decrease CH_4 emissions as outlined by comprehensive reviews (Hristov et al., 2013; Beauchemin et al., 2020). Among the strategies proposed, the investigational CH_4 inhibitor 3-nitrooxypropanol (3-NOP; DSM Nutritional Products Ltd., Kaiseraugst, Switzerland) shows tremendous promise with 20–80% decreases in CH_4 production depending upon the type of animal, diet composition, dose and method of supplementation (Hristov et al., 2015; Vyas et al., 2016a, 2018; Dijkstra et al., 2018; McGinn et al., 2019). This inhibitor reduces methanogenesis in the rumen by inactivating the enzyme methyl-coenzyme M reductase used by archaea (Duin et al., 2016). The decrease in CH_4 production was shown to persist over several months when 3-NOP was included in the diets of lactating dairy cows (25–32% decrease, 12-week study; Hristov et al., 2015) and growing beef cattle (37–42% decrease over 238 d; Vyas et al., 2018).

Incorporating 3-NOP into beef cattle diets to decrease CH_4 production could allow producers to participate in the carbon market, such as the carbon pricing system in Canada (Government of Canada, 2019), by trading CO_2 equivalents. Additionally, any improvement in animal performance [body weight (BW) gain, feed conversion efficiency] resulting from feeding 3-NOP could help offset the additional costs of using the feed additive. Therefore, it is important to evaluate, at a commercial scale, the results for CH_4 mitigation and animal performance reported in controlled research studies that evaluated 3-NOP. Previous beef cattle studies were conducted using individually housed animals (Romero-Perez et al., 2014, 2015) or small pens (≤10 cattle/pen) of animals (Vyas et al., 2016a,b, 2018) that minimize social interactions among animals and thus do not reflect the conditions of commercial feedlots where animals are housed in large groups (>100 cattle/pen). Thus, the objectives of this study were to evaluate the effects of feeding 3-NOP to backgrounded beef cattle on feed consumption, animal performance, animal health and enteric CH_4 production in a commercial feedlot setting.

MATERIALS AND METHODS

Research Overview, Site Description, and Diet

Two concurrent studies were conducted using procedures in accordance with the guidelines of the Canadian Council on Animal Care (2009) and with approval of the Veterinary Drug Directorate of Health Canada (DSTS No. 207171). The animal performance study evaluated the effects of 3-NOP supplementation on dry matter intake (DMI), average daily gain (ADG), feed conversion efficiency (gain:feed ratio, G:F), and health using cattle housed in 16 commercial feedlot pens. The effects of 3-NOP on enteric CH_4 production were evaluated in a second study using a subset of cattle maintained in two small pens. The animal performance study was conducted between November 2017 and September 2018 for a total of 108 ± 8 d (12 ± 3 d of adaptation, 89 ± 8 d at the final concentration for measurements), while the CH_4 production study was conducted between May and September, 2018 for a total of 112 d (28 d of adaptation, 84 d of measurement).

The research was conducted at a commercial beef cattle feedlot located near Nanton, Alberta, Canada. The basic design of the feedlot is representative of standard designs used in western Canada where animals are housed in open-air, dirt-floor pens arranged side-by-side with central feed alleys and 20% porosity wood-fence windbreaks. The 16 large pens used in the performance study had dimensions of ∼61 m × 70 m with a capacity of ∼250 animals per pen and were equipped with a concrete feed bunk along one side of the pen. The two small pens used in the CH_4 production study had dimensions of ∼15.5 m × 29.9 m with a capacity of 25 animals per pen and were equipped with four electronic feed bunks (GrowSafe, Calgary, Alberta, Canada) per pen. One of the pens was equipped with a GreenFeed emission monitoring (GEM) system (C-Lock Inc., Rapid City, SD, USA). There were three animal handling facilities located at the site. Each facility contained a hydraulic chute equipped with an individual animal scale, a chute-side computer with individual animal data collection and management software (*i*FHMS©; Feedlot Health Management Systems, Okotoks, Alberta, Canada), and separation alleys to facilitate the return of animals to designated pens. Open-air containment pens are located adjacent to each facility.

A total of 4,048 mixed breed steers were assigned to the two concurrent studies. In the large pen study, the cattle were fed a backgrounding diet containing 70% whole crop barley (*Hordeum vulgare* L.) silage and 30% barley-based concentrate [dry matter (DM) basis; **Table 1**]. In the small pen study, the initial diet formulation contained barley silage but due to an unexpected shortage, corn silage (*Zea mays*) was used mid-way (starting August 19, 2018) until the end of the experiment. Micro-ingredients (ionophore, antibiotic, minerals, vitamins, and 3-NOP) were added to the ration via a water-based micro-ingredient machine. The ionophore monensin (Rumensin, Elanco Canada Limited, Guelph, Ontario, Canada) was included at 25 mg/kg DM and chlortetracycline (Chlor 100, Bio-Agri Mix, Mitchell, Ontario, Canada) was included at 35 mg/kg DM for liver abscess control. The 3-NOP was a dry granular product with an active ingredient concentration of 100 g 3-NOP/kg. The rations were mixed thoroughly before delivery into the feed bunks, and prior to starting the experiment, the mixer underwent testing to validate the weigh scale and mixing consistency.

Animal Performance and Health Study

The animal performance and health study was conducted in large pens (average 253 animals/pen; range: 244–263 animals/pen), with eight replicates. Each replicate was assigned to two pens; control and 3-NOP, and thus the experimental unit was the pen ($n = 8$/treatment group). Animals (mixed breed beef steer calves) for the study were sourced from various auction markets across Canada and randomly allocated to treatments upon arrival to the feedlot or at re-handling (39 d after arrival). The average (±SD) initial individual animal live weight of study pens was 283 ± 9 and 282 ± 8 kg for the control and 3-NOP groups, respectively. Animals in the control group (2,025 animals) were fed the backgrounding basal diet without 3-NOP whereas animals in the 3-NOP group (2,023 animals) were fed the same basal diet containing 3-NOP at a concentration of 200 mg/kg DM basis. Animals in the 3-NOP group were incrementally adapted to reach the final concentration of 3-NOP: 100 mg 3-NOP/kg DM for 7–10 d, 150 mg 3-NOP/kg DM for 7 d, and the final concentration of 200 mg/kg DM until the end of the study. The dose of 3-NOP was based on previous studies including Vyas et al. (2016a,b, 2018) and Romero-Perez et al. (2014, 2015).

Feed was delivered twice daily (and topped-up throughout the day when needed) by mixer trucks equipped with load cells. Feed bunks were monitored every day before morning feeding and feed adjustment was made based on slick-bunk management (Schwartzkopf-Genswein et al., 2003; Schutz et al., 2011). Daily DMI was calculated as the total feed delivered to the pen daily, adjusted for DM content, divided by the number of animals in the pen. As minimal feed remained in the feed bunk prior to the next day's feed allocation, it was not necessary to account for feed refusals.

Animal health was monitored by experienced herdsmen and veterinarians. The herdsmen conducted health monitoring on a daily basis and used subjective criteria based on modified DART system (Depression, Appetite, Respiratory signs and Temperature; Step et al., 2008) for identification and further evaluation and treatment of sick animals. Animals were weighed before feeding (non-fasted) once at the start and once at the end of the study to determine body weight (BW) gain. The weight gain was divided by number of days on the study to calculate ADG (shrunk), and feed conversion efficiency was measured as G:F (kg:kg) ratio by dividing ADG by daily DMI.

TABLE 1 | Ingredient inclusion rate and diet composition (mean ± SD) for the basal diet used in the animal performance and methane production studies.

Item	Basal diet[a]
Diet ingredients, % of DM[b]	
Barley silage or corn silage	70.14
Steam-flaked barley grain	28.74
Supplement[c]	1.12
Diet composition, % of DM	
DM, % as-fed	39.4 ± 2.26
Organic matter	92.2 ± 0.40
NDF	44.0 ± 3.24
ADF	22.0 ± 1.59
CP	12.0 ± 1.96
Gross energy, MJ/kg DM	21.4 ± 0.67

[a]*Combined analysis of control diet and diets containing 3-NOP.*
[b]*Barley silage contained (DM basis): 53.2 ± 1.47% NDF, 28.4 ± 2.21% ADF, 13.7 ± 1.15% CP, and 21.8 ± 0.56 MJ/kg gross energy; corn silage contained (DM basis): 51.7 ± 1.02% NDF, 28.4 ± 0.80% ADF, 8.6 ± 0.79% CP, and 21.8 ±0.17 MJ/kg gross energy; steam-flaked barley grain contained (DM basis): 20.5 ± 2.99% NDF, 5.5 ± 0.60% ADF, 12.3 ± 1.02% CP, and 20.9 ± 0.58 MJ/kg gross energy.*
[c]*Includes: Limestone, vitamin-trace mineral premix, monensin sodium (to provide 25 mg/kg; Rumensin, Elanco Canada Limited, Guelph, Ontario, Canada), chlortetracycline (to provide 35 mg/kg; Chlor 100, Bio-Agri Mix, Mitchell, Ontario, Canada).*

Methane Production Study

A subset of animals allocated to one of the replicates (Replicate 8) in the animal performance and health study (80 animals, 40 from each experimental group) were removed from the large pens at the start of the study, weighed over two consecutive days (non-fasted), and ranked by BW. Twenty-five candidate animals from each group were then selected based on BW to provide two balanced groups for the CH_4 production study. The remaining 15 animals from each experimental group were returned to the appropriate large pen. The selected animals (initial BW = 328 ± 29 kg) were maintained on their respective experimental group assignments throughout the study: control and 3-NOP. Prior to starting the study, the animals in the 3-NOP group received the backgrounding diet containing 100 mg 3-NOP/kg DM for 7 d. Once assigned to the CH_4 production study, they were provided a 21-d adaptation (adaptation phase) during which they continued to receive the diet containing 150 mg 3-NOP/kg DM. The adaptation phase allowed the animals to become familiar with the CH_4 emission monitoring system described below. Following the adaptation phase, the study was conducted in three 28-d phases, with the concentration of 3-NOP in the basal diet increased in each phase: low (150 mg/kg DM) in Phase 1, medium (175 mg/kg DM) in Phase 2, and high (200 mg/kg DM, final dose) in Phase 3. An additional 2 weeks of adaptation was added between Phases 1 and 2, during which barley silage was replaced with corn silage due to a shortage of barley silage at the feedlot. Animals were fed twice a day (0900 and 1,500 h) for *ad libitum* intake (5% orts). Orts were removed and weighed weekly. Because individual animal data were obtained for gas measurements and DMI, the experimental/observational unit was the animal (Bello and Renter, 2017).

Emission Measurements

Methane and H_2 were measured using the GEM system. Each week the two pens of animals were rotated, such that each pen of cattle had access to the GEM system for 1 week, every second week. This approach controlled any possible pen effects and has been implemented previously (Alemu et al., 2019, 2020). Within a phase, each pen had access to the system for two 7-d periods.

The GEM system allows free movement of animals (in and out of the system) and gasses are measured only when the animal's head is in the "head chamber" unit as determined by the proximity sensor. The system is equipped with a radio-frequency reader that identifies the electronic ear tag of each animal. During a visit to the GEM system, animals are provided with pellets from an overhead hopper (as bait). The interval between pellet drops was set to 35 s to keep the animals in the hood for sufficient time (3–7 min) to obtain a full measurement. Maximum daily pellet drops per animal (36 drops, 6 drops/visit) was set to restrict the amount of pellet consumption. The total amount of pellet DM consumed per animal per day was added to the animal's intake of basal diet DM from the GrowSafe bunks, to calculate total DMI. Animals could visit the system any time but they were eligible for pellet drops only during six visits that were spaced a minimum of 4 h apart during each 24-h cycle. Thus, animals were required to wait 4 h before getting their next pellet drop. The pellet was composed of ground barley, canola meal and oil, dried molasses and salt with a composition of (% of DM): 14.4 ± 1.10 crude protein (CP), 22.0 ± 1.62 neutral detergent fiber (NDF), 8.23 ± 0.48 acid detergent fiber (ADF), and 20.5 ± 0.29 MJ/kg gross energy.

Once the animal's head is in the hood of the GEM system, air is drawn past the nose and mouth of the animal at about 26–40 L/s into the collection pipe. The system measures CH_4, CO_2, and H_2 continuously together with air flow, temperature, atmospheric pressure, and relative humidity. Each gas is analyzed by a separate non-dispersive infrared analyzer, which was calibrated weekly using a zero (semipure nitrogen) and span CH_4 and CO_2 gases, with nitrogen as the balance gas. The purpose of the calibration was to define sensor responses to known concentration of gasses. Five times during the experiment, the air flux sensor was calibrated by releasing a gravimetrically determined quantity of CO_2 into the system using a 90-g prefilled CO_2 cylinder for 3 min (at least three times). The amount released was compared to the calculated capture (96.7% CO_2 recovery, SD = 4.6, $n = 5$). To maintain a consistent airflow rate between 27 and 40 L/s in the collection tube (Velazco et al., 2016), the air filter was cleaned and changed regularly (every 3–5 days).

To calculate mass flux of CH_4, the measured increase in concentration in the animal's breath relative to that in the ambient (background) air was multiplied by the measured air flow rate, and then the ideal gas law was applied.

$$CH_{4-volume} = F_c * \frac{1}{C_R} * \sum_{tp} [\Delta t * (CH_{4-average} - CH_{4-background}) * Q_{air}]$$

Where, F_C is the dimensional factor; $1/C_R$ is the capture rate of emissions into collection pipes determined using a tracer (%); Δt is time period over which emissions are measured (1 s); $CH_{4-average}$ is average concentration of CH_4 (%); $CH_{4-background}$ is background concentration of CH_4 (%); and Q_{air} is air flow rate (flow per unit time). Once the volume flow rates of CH_4 are determined, the mass flux is determined by applying the ideal gas law. Daily CH_4 emission for individual animals was calculated over the 7-d by aggregating and averaging the visit fluxes by time of day (for each 4-h block). Within each phase, only cattle with ≥10 useful/good visits with visits in at least five of the six 4-h time blocks were used in the analysis to ensure that the full diurnal cycle of emission was represented, as CH_4 emissions fluctuate over the 24-h cycle (Gunter and Bradford, 2015; Manafiazar et al., 2016). Hydrogen was calculated using an "arithmetic averaging method," which is a straight averaging of the visit fluxes (Manafiazar et al., 2016).

Sample Collection and Analysis

Feed ingredients and the basal diet offered were sampled every day during the CH_4 emission study and composited by week for further chemical analyses. A sample of pellets from the GEM system was collected every 2 weeks for chemical analysis. The chemical composition of ingredients and basal diet are presented in **Table 1**. Feed ingredients, basal diet and pellet samples were dried at 55°C for 72 h in a forced air oven for DM determination. Dried samples were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) through a 1-mm screen. Analytical DM content of the ground samples was determined by drying at 135°C for 2 h (method 930.15; AOAC, 2016). The NDF and ADF contents were determined sequentially using an Ankom A200 fiber analyzer (Ankom Technology, Macedon, NY), with heat-stable amylase and sodium sulfite used for NDF analysis. Gross energy concentration was determined using a bomb calorimeter (model E2k, CAL2k, Johannesburg, South Africa). Samples ground through a 1-mm screen were reground using a ball grinder (Mixer Mill MM2000; Retsch GmbH, Haan, Germany) before determination of nitrogen content. The nitrogen content (CP = nitrogen × 6.25) was determined by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy). Ground, dried samples of the basal diet were shipped to DSM Nutritional Products (Basel, Switzerland) for measurement of 3-NOP concentration using HPLC (method AP.227089.01) and propanediol mononitrate as a reference standard.

Statistical Analysis

For the large pen study, the animal performance data (DMI, ADG and G:F) for each pen were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) where experimental group (control, 3-NOP) was considered a fixed effect and replicate (1–8) a random effect. Morbidity and mortality data

TABLE 2 | Performance of beef steers fed a backgrounding basal diet supplemented with and without 3-nitrooxypropanol (3-NOP; 200 mg/kg DM; $n = 8$ pens/treatment).

Item	Control	3-NOP	SEM	*P*-value
Start BW, kg	283	282	3.01	0.62
End BW, kg	422	421	6.11	0.70
Total weight gained, kg	139	139	3.74	0.87
ADG, kg/d	1.30	1.30	0.06	0.87
DMI, kg/d	8.07	7.86	0.26	0.06
G:F	0.16	0.17	0.003	0.06

aG:F = Gain:Feed ratio calculated as kg live weight gain/kg dry matter intake.

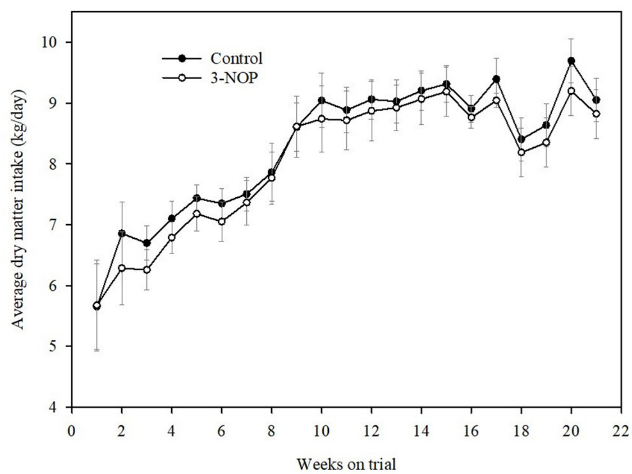

FIGURE 1 | Average dry matter intake of steers in the animal performance study fed a backgrounding diet supplemented without (control) or with 3-nitrooxypropanol (3-NOP) at 200 mg 3-NOP/kg DM over the experimental period. Animals had *ad libitum* access to feed. Error bars indicate standard error.

TABLE 3 | Mortality and post-allocation morbidity percentages for beef steers fed a backgrounding basal diet supplemented with and without 3-nitrooxypropanol (3-NOP; 200 mg/kg DM; $n = 8$ pens/treatment).

Item	Control	3-NOP[a]	*P*-value
Morbidity, %			
Initial undifferentiated treatment of fever	2.24	2.25	0.99
First undifferentiated fever relapse treatment[b]	17.63	28.57	0.72
Initial treatment without fever	4.05	3.38	0.64
First relapse treatment without fever[c]	10.82	15.12	0.07
Chronicity (chronic disease, all causes)	0.69	0.69	0.99
Wastage[d]	0.34	0.64	0.13
Mortality, %			
Overall	0.49	0.69	0.32
Respiratory disease	0.15	0.20	0.67
Lesions consistent with *Histophilus Somni* infection	0.20	0.10	0.33
Metabolic disease	0.14	0	NA
Lameness	0	0.05	NA
Other	0	0.35	NA

[a]*No health issues was observed for animals used in the methane production study in small pens.*
[b]*Number of animals treated for first undifferentiated relapse following allocation divided by the number of animals treated for initial undifferentiated relapse.*
[c]*Number of animals treated for first no-fever relapse following allocation divided by the number of animals treated for initial no-fever.*
[d]*Wastage is the number of animals with chronic disease (all causes) that did not die divided by the number of animals allocated.*
NA, not available.

were analyzed using the GENMOD procedure in SAS where experimental group was considered a fixed effect and pen within replicate as a clustering effect.

For the small pen CH_4 study, DMI and gas data for each animal were analyzed by phase and overall using the MIXED procedure of SAS. Within phase, the model included experimental group (control, 3-NOP), week (1–4 for DMI, 1 and 2 for gases), and their interaction as fixed effects, with week considered as a repeated effect in the model. Kenward-Roger's option was used in the model statement to estimate denominator degrees of freedom. Best time-series covariance structure was selected on the basis of the lowest Akaike and Bayesian information criteria and compound symmetry was used. Residual plots were used to check the validity of the underlying statistical assumptions of homogeneity of variances and normality. Statistical significance was declared at $P < 0.05$ and trends are discussed at $P \leq 0.10$.

RESULTS

Animal Performance Study

Inclusion of 3-NOP tended to decrease DMI (2.6%, $P = 0.06$), without affecting total weight gain ($P = 0.87$) or ADG ($P = 0.87$) (**Table 2**). As a result, G:F tended to improve (2.5%; $P = 0.06$) by feeding 3-NOP compared with the control (0.16 kg liveweight gain/kg DMI). The pattern of DMI over the study showed that the cattle fed 3-NOP generally had lower intake during the first 6 weeks of the feeding period (**Figure 1**). There were no differences in morbidity or mortality detected between the experimental groups (**Table 3**). However, there was a tendency for animals fed 3-NOP to have a slightly greater percentage ($P = 0.07$) of treatment of animals for relapse without a fever.

Methane Production Study

The measured concentrations of 3-NOP in the basal diet offered in the GrowSafe bunks and calculated concentrations in the total feed consumed (basal diet plus pellet offered in the GEM system) during the study are presented in **Table 4**. Recovery of 3-NOP in the basal diet averaged 105.7%, ranging from 89.7 to 120.7%, which is within an acceptable range for most feed additives. Calculated concentration of 3-NOP in the total DMI, which accounts for intake of the basal diet and the pellets offered in the GEM system, was 124.6, 192.8, and 226.8 mg/kg DM for the low,

TABLE 4 | Targeted and measured concentration of 3-nitrooxypropanol (3-NOP) in the diets fed during the methane emission measurement study ($n = 4$ observations/phase, mean ± SD).

Item[a]	Phase			
	Adaptation[b]	Low	Medium	High
Target concentration in basal diet, mg 3-NOP/kg DM	150	150	175	200
Measured concentration in basal diet, mg 3-NOP/kg DM	141.1 ± 11.8	134.5 ± 25.4	207.2 ± 13.6	241.4 ± 8.3
Recovery, %[c]	94.1	89.7	118.4	120.7
Calculated concentration in total diet consumed, mg 3-NOP/kg DM	130.8	124.6	192.8	226.8
Intake, g 3-NOP/d[d]	1.11 ± 0.167	1.27 ± 0.291	2.25 ± 0.266	2.75 ± 0.372

[a] *Basal diet was provided in the GrowSafe feed bunks and total diet refers to basal diet + pellets provided in the GreenFeed system. 3-NOP concentration in the control diet was zero.*
[b] *Animals adapted to 3-NOP and the GreenFeed emission monitoring system for 28 d.*
[c] *Calculated as: (measured 3-NOP concentration/target concentration) × 100.*
[d] *Daily 3-NOP intake was calculated from the measured concentration in the basal diet and the measured total DMI of each animal.*

TABLE 5 | Dry matter intake (DMI, kg/d; basal diet, pellet, and total) for beef steers ($n = 25$) during the enteric CH_4 emission measurement study.

Phase[a]	Control	3-NOP	SEM	*P*-value
Phase 1: Low dose				
Total diet[b]	9.64	9.38	0.25	0.31
Basal diet (GrowSafe system)	9.37	9.02	0.27	0.19
Pellet (GreenFeed system)	0.27	0.36	0.06	0.10
Phase 2: Medium dose				
Total diet[b]	11.55	10.88	0.27	0.02
Basal diet (GrowSafe system)	11.25	10.48	0.28	0.01
Pellet (GreenFeed system)	0.30	0.39	0.04	0.04
Phase 3: High dose				
Total diet[b]	12.06	11.35	0.37	0.06
Basal diet (GrowSafe system)	11.76	10.98	0.39	0.05
Pellet (GreenFeed system)	0.30	0.37	0.04	0.12
Overall: Phases 1–3				
Total diet[b]	10.43	9.87	0.23	0.02
Basal diet (GrowSafe system)	10.16	9.51	0.25	0.01
Pellet (GreenFeed system)	0.27	0.36	0.05	0.07

Steers were fed a backgrounding basal diet without (control) or with increasing doses of 3-nitrooxypropanol (3-NOP).
[a] *Low dose = 150 mg 3-NOP/kg DM, medium dose = 175 mg 3-NOP/kg DM, high dose = 200 mg 3-NOP/kg DM. 3-Nitrooxypropanol was added only to the basal diet.*
[b] *Total dietary DMI is the sum of DMI of the basal diets delivered in the GrowSafe system and DMI of the pellet delivered in the GEM system.*

medium and high doses respectively. Average daily consumption of 3-NOP increased with inclusion rate as expected, 1.27 g/d for the low, 2.25 g/d for medium, and 2.75 g/d for the high dose.

Dry matter intake for the low (phase 1), medium (phase 2), and high (phase 3) doses of 3-NOP is reported in **Table 5**. On average, total DMI was lower ($P = 0.02$) by 5.3% for 3-NOP as compared to the control treatment (10.43 kg/d). Intake was not affected by the low dose but was 5.8% less for the medium dose compared with control (11.55 kg/d) and 5.9% lower for the high dose compared with control (12.06 kg/d). For animals visiting the GEM system, average pellet consumption tended to be greater ($P = 0.07$) for the 3-NOP group (0.36 kg/d) relative to the control group (0.27 kg/d).

Of the 25 animals assigned to each treatment, on average, 76% of animals (ranging between 60 and 84%) for control and 88% of animals (ranging between 80 and 92%) for 3-NOP visited the GEM system (**Table 6**). Over the study period, the average total visits were 1,156 and 1,556 for control and 3-NOP groups, respectively. The number of weekly visits to the GEM system and the number of 4-h blocks in which visits occurred were not affected by treatment group in any phase ($P \geq 0.20$). The visits to the GEM system were relatively consistent throughout the 24-h period, with the exception of between 0300 and 0400 h (**Figure 2**). On average, each visit to the GEM system lasted slightly more than 4 min and did not differ ($P \geq 0.48$) between treatment groups (**Table 6**).

TABLE 6 | Visits[a] to the GreenFeed emission monitoring (GEM) system for beef steers fed a backgrounding basal diet without (control) or with increasing doses of 3-nitrooxypropanol (3-NOP).

Phase: Dose[b]	Control	3-NOP	SEM	*P*-value
Phase 1: Low dose				
No. of animals visiting the GEM system	15	20–21	…	…
No. of 4-h block in which visits occurred	6.0	6.0	0.0	…
Weekly visits per animal	35.1	40.2	3.62	0.22
Duration (min:s)	4:33	4:38	0.13	0.70
Phase 2: Medium dose				
No. of animals visiting the GEM system	19–21	22–23	…	…
No. of 4-h block in which visits occurred	5.95	5.98	0.06	0.64
Weekly visits per animal	31.4	34.9	1.93	0.20
Duration (min:s)	4:19	4:09	0.13	0.48
Phase 3: High dose				
No. of animals visiting the GEM system	19–21	23	…	…
No. of 4-h block in which visits occurred	5.97	5.98	0.04	0.91
Weekly visits per animal	31.0	32.1	1.82	0.61
Duration (min:s)	4:43	4:58	0.25	0.57
Overall: Phases 1–3				
No. of animals visiting the GEM system	18–19	22	…	…
No. of 4-h block in which visits occurred	5.97	5.98	0.02	0.61
Weekly visits per animal	32.1	35.4	2.09	0.20
Duration (min:s)	4:17	4:13	0.09	0.64

[a] *Visits were compiled into six 4-h blocks corresponding to time of day. Only "useful/good" visits of at least 3-min were used to calculate weekly visits per animal (Arthur et al., 2017; Beck et al., 2018). Only animals with ≥10 "useful/good" weekly visits, with visits in at least 5 of the six 4-h time blocks were selected for final analysis.*

[b] *Low dose = 150 mg 3-NOP/kg DM, medium dose = 175 mg 3-NOP/kg DM, high dose = 200 mg 3-NOP/kg DM. 3-Nitrooxypropanol was added only to the basal diet.*

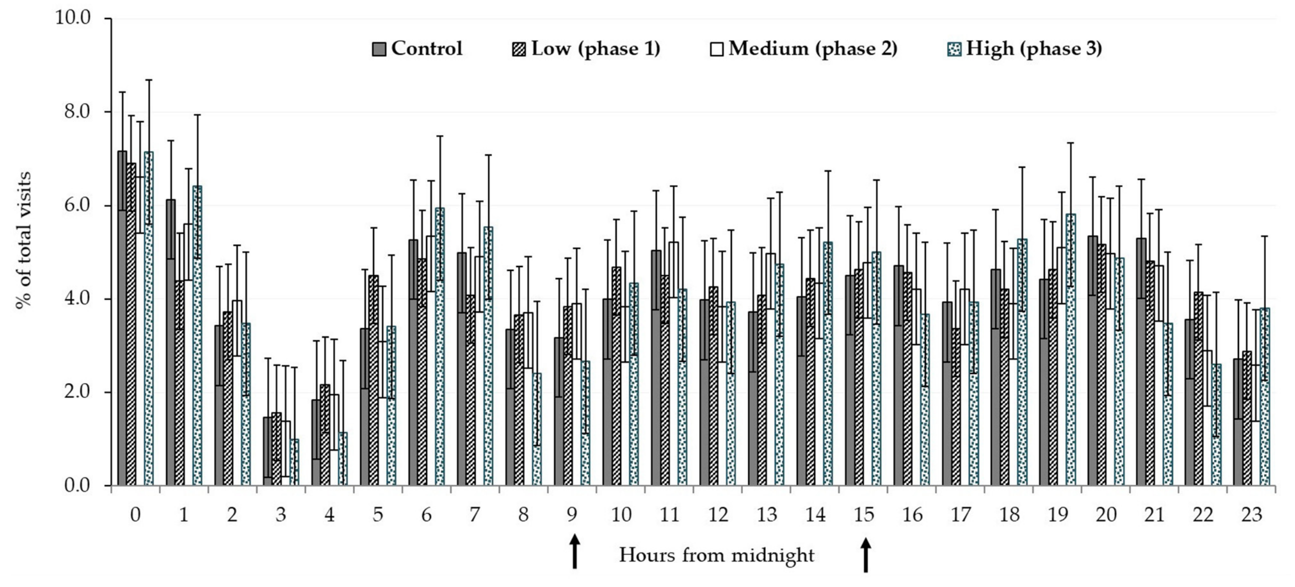

FIGURE 2 | Average diurnal pattern of animal visits to the GreenFeed emissions monitoring system for beef steers in the methane production study fed a backgrounding diet without (control) and with 3-nitrooxypropanol (3-NOP) supplemented at increasing concentrations in the basal diet: low (150 mg 3-NOP/kg DM, Phase 1), medium (175 mg 3-NOP/kg DM, Phase 2), and high (200 mg 3-NOP/kg DM, Phase 3). Arrows indicate time of feeding at 0900 and 1500 h and error bars indicated standard error. Data are averaged for all animals that visited the GEM system.

TABLE 7 | Enteric methane (CH_4) and hydrogen (H_2) emissions for beef steers fed a backgrounding basal diet without (control) or with increasing doses of 3-nitrooxypropanol (3-NOP).

Phase: Dose[a]	Emission[b]	Units	Control	3-NOP	SEM	*P*-value
Phase 1: Low dose	CH_4	g/d	224.8	185.7	8.04	0.01
		g/kg total DMI	23.76	18.97	1.16	0.01
		% gross energy intake	5.99	4.85	0.30	0.01
	H_2	g/d	0.37	1.29	0.09	<0.001
		g/kg total DMI	0.04	0.14	0.01	<0.001
Phase 2: Medium dose	CH_4	g/d	259.7	184.9	6.18	<0.001
		g/kg total DMI	22.33	16.64	0.61	<0.001
		% gross energy intake	5.82	4.43	0.16	<0.001
	H_2	g/d	0.41	1.64	0.08	<0.001
		g/kg total DMI	0.03	0.14	0.01	<0.001
Phase 3: High dose	CH_4	g/d	275.7	198.4	8.51	<0.001
		g/kg total DMI	22.40	17.68	0.62	<0.001
		% gross energy intake	5.88	4.80	0.17	0.002
	H_2	g/d	0.39	1.55	0.06	<0.001
		g/kg total DMI	0.03	0.13	0.01	<0.001
Overall: Phases 1 to 3	CH_4	g/d	255.2	189.6	6.91	0.001
		g/kg total DMI	22.49	17.61	0.64	0.001
		% gross energy intake	5.89	4.67	0.16	0.001
	H_2	g/d	0.39	1.50	0.05	<0.001
		g/kg total DMI	0.03	0.14	0.01	<0.001

DMI, dry matter intake.

[a]*Low dose = 150 mg 3-NOP/kg DM, medium dose = 175 mg 3-NOP/kg DM, high dose = 200 mg 3-NOP/kg DM. 3-Nitrooxypropanol was added only to the basal diet.*

[b]*Only animals with ≥ 10 "useful/good" weekly visits, with visits in at least 5 of the six 4-h time blocks were selected for CH_4 analysis.*

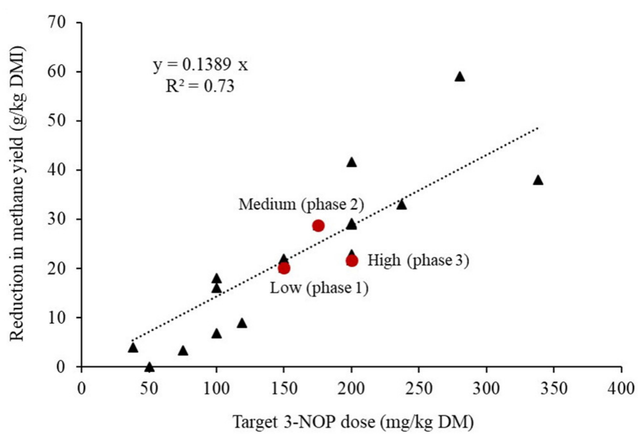

FIGURE 3 | Reduction in methane yield (g CH_4/kg dry matter intake, DMI) observed in the study (indicated by closed circles) compared with the published literature (indicated by closed triangles) for feedlot cattle fed high-forage backgrounding diets. The linear regression line is fitted through all the data using an intercept of zero. Literature studies were Alemu et al. (present study, unpublished), Romero-Perez et al. (2014), Vyas et al. (2016a,b, 2018), Smith (2017), Martinez-Fernandez et al. (2018), and Kim et al. (2019).

Methane and H_2 production (g/d) and yield (g/kg DMI) for the low (phase 1), medium (phase 2), and high (phase 3) doses of 3-NOP are reported in **Table 7**. Methane production (g/d) decreased ($P \leq 0.01$) by 17.4, 28.8, and 28.1% for the low, medium and high doses of 3-NOP, respectively, compared with control, averaging 255.2 g/d. Thus, over the entire study, 3-NOP reduced CH_4 production by an average of 25.7% ($P = 0.001$). Methane yield (g/kg DMI) followed a similar pattern with decreases of 17.2, 25.7, and 21.3% for low, medium and high doses of 3-NOP, respectively, with a 21.7% decrease overall ($P = 0.001$). When CH_4 energy was expressed as percentage of gross energy intake, feeding 3-NOP decreased emissions by 19.1, 23.9, and 18.4% for the three doses respectively, and by 20.8% overall ($P \leq 0.01$). The reduction in CH_4 yield by phase is compared with previous literature for beef cattle fed high-forage diets in **Figure 3**.The average diurnal pattern of CH_4 emissions during the study by treatment is presented in **Figure 4A**.

With the decrease in CH_4 production due to 3-NOP, there was a concomitant increase in H_2 production, which increased from 0.39 g/d for control to 1.50 g/d ($P < 0.001$) for 3-NOP overall. The increases in H_2 emissions were consistent within each phase. The average diurnal pattern of H_2 production during the study by treatment is presented in **Figure 4B**.

DISCUSSION

Animal Performance and Health

Two previous controlled research studies conducted in small pens examined the effects of including 3-NOP in high-forage backgrounding diets on animal performance (Vyas et al., 2016a, 2018). In a 105-day study, Vyas et al. (2016a) reported that

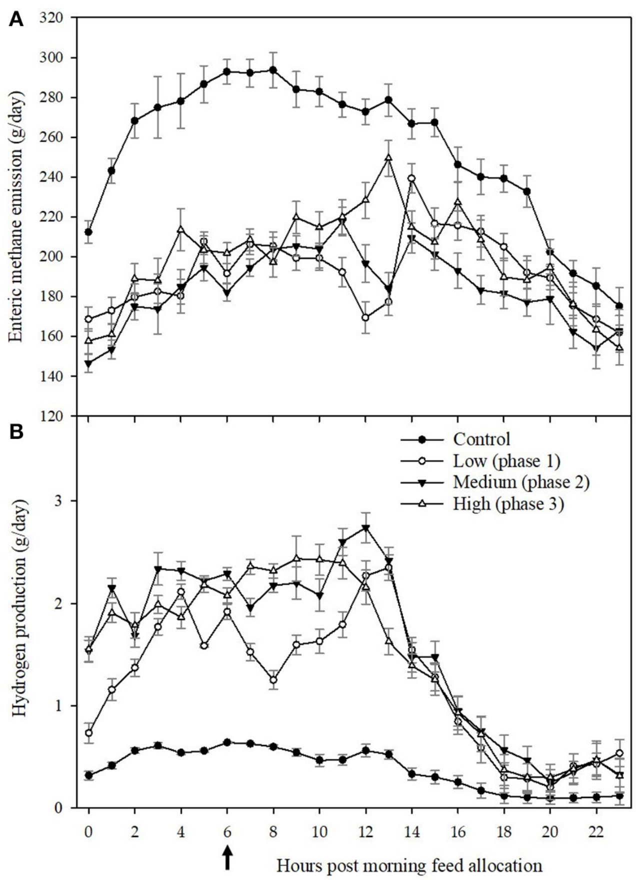

FIGURE 4 | Mean hourly enteric methane emissions **(A)** and hydrogen production **(B)** from feedlot steers in the methane production study fed a backgrounding diet without (control) and with 3-nitrooxypropanol (3-NOP) supplemented at increasing concentrations in the basal diet: low (150 mg 3-NOP/kg DM, Phase 1), medium (175 mg 3-NOP/kg DM, Phase 2), and high (200 mg 3-NOP/kg DM, Phase 3). Animals were fed twice a day at 0900 h (indicated as 0) and 1500 h (indicated by the arrow), and error bars indicated standard error.

inclusion of 3-NOP (200 mg/kg DM) reduced DMI by 8.0% and increased G:F by 7.7%. Using the same concentration of 3-NOP, Vyas et al. (2018) reported 9.2% reduction in DMI and 6.5% improvement in G:F for beef steers relative to control. Several factors including improved energy status of the animal as a result of decreased energy loss in the form of CH_4 and a shift in metabolic precursors may account for the observed reduction in DMI and improved efficiency (Lee et al., 2019; Alemu et al., 2020). The present study is the first to examine the effects of 3-NOP in a commercial feedlot setting in which animals were fed a backgrounding diet and group-housed in large pens that are representative of the scale that exists in commercial production scenarios, which may create considerable competition among animals at the feed bunk. For beef cattle fed forage-based diets, Custodio et al. (2017) reported that feeding behavior of cattle can be influenced by housing system (individual vs. collective pen). Before beef producers use 3-NOP to mitigate CH_4 emissions they need information on the health and performance of animals fed 3-NOP in commercial conditions, in addition to efficacy of CH_4 reduction.

Using a 3-NOP concentration of 200 mg/kg DM in the performance study reduced DMI by 2.6%, which was considerably less than that observed in previous small-scale research studies (Vyas et al., 2016a, 2018). The relatively small decrease in DMI of animals in the performance study (2.6%) also contrasts with the observed 5.9% reduction in DMI of animals in the CH_4 study, when fed the high dose (200 mg 3-NOP/kg DM). The greater decline in DMI of cattle fed 3-NOP in small-pen studies does not appear to be due to lack of adaptation of animals to 3-NOP, because in the present studies the animals were gradually transitioned to 3-NOP. Furthermore, during CH_4 measurements, for which the reduction in DMI was greatest, the transition was gradual with use of an adaption period followed by a phased step-up. As DMI relative to BW was similar for the control animals in both the performance and CH_4 studies, the reasons for the greater decline in DMI of animals fed 3-NOP in the small pens are not clear. It is possible that the differing response is related to differences in competition at the feed bunk caused by the type of feeder (long trough in the large pens vs. feed bins in the small pens), feed bunk management (slick-bunk vs. ad lib, Schwartzkopf-Genswein et al., 2003; Schutz et al., 2011) and the frequency of feed allocation (top-up throughout the day in large pens vs. 2-times daily in small pens). The smaller decline in DMI observed in the animal production study may account for the relatively smaller (2.5%) improvement in feed conversion efficiency compared with the 6.5–7.7% improvement observed in previously reported small-pen studies (Vyas et al., 2016a, 2018).

Animal health in response to 3-NOP supplementation has not been previously documented. Although the finding of no increased risk of mortality and morbidity in cattle fed 3-NOP has important implications for future use of the product by commercial feedlots, the observed tendency for the treatment of animals with relapse without a fever requires further investigation.

Gaseous Emissions

A meta-analysis of data from 11 experiments (Dijkstra et al., 2018) indicated that with a mean inclusion rate of 123 mg 3-NOP/kg DM, enteric CH_4 production (g/d) and yield (g/kg DM) were reduced by 22.2 and 17.1%, respectively, in beef cattle fed a range of diets. Those authors also indicated that the effect of 3-NOP on enteric CH_4 production was positively associated with dose and inversely associated with diet NDF concentration. Thus, the response to 3-NOP at a particular level of 3-NOP would be expected to be less for backgrounded cattle fed higher fiber diets compared with finishing cattle fed grain-based diets. The reduction in CH_4 yield observed in the present study (decrease of 20.1, 25.5, and 21.1% for low, medium and high doses of 3-NOP; 21.7% decrease overall) is consistent with the previous literature (**Figure 3**), although a linear response to 3-NOP concentration was not observed in the present study. When examined over nine beef studies (including the present study) with 17 treatment means of cattle fed high-forage diets ($\geq$60% forage DM), the linear response to targeted dietary concentration of 3-NOP is: Reduction in CH_4 yield = 0.1389 × mg 3-NOP/kg DM (R^2 = 0.73). The reason for the lack of linear response to 3-NOP dose in the present study is not clear. One factor may be the study design wherein each dose of 3-NOP was evaluated sequentially, rather than simultaneously, and hence the cattle were exposed to 3-NOP for a differing number of days at each dose, making it difficult to compare the animal responses across dose.

Methane is the largest sink of H_2 in the rumen. When methanogenesis is inhibited gaseous H_2 can accumulate (Hristov et al., 2015; Vyas et al., 2018), as was observed in the present study where H_2 was on average 1.50 g/d for the 3-NOP group compared with 0.39 g/d for control cattle. Although the release of gaseous H_2 represents an inefficiency of energy utilization, the loss of energy as H_2 was only 4% of the energy potentially available from the decrease in CH_4 production (65.7 g CH_4, 55 kJ/g vs. 1.11 g H_2, 142 kJ/g). In terms of reducing equivalents, 65.7 g/d of spared CH_4 is equivalent to releasing 16 g/d H_2. It appears that <2% of the spared H_2 was released as gas, indicating that >98% was diverted toward dissolved H_2 and alternate H_2 sinks in the rumen (e.g., such as formate, propionate, valerate, caproate, heptanoate, unsaturated fatty acids, nitrate and sulfate reduction, and microbial protein synthesis; Guyader et al., 2017). This shift in H_2 flow within the rumen toward nutritionally beneficial sinks may partially account for the observed improvement in G:F.

Implications for Sustainable Beef Production

Approximately 80% of Nationally Determined Contributions to meet the commitments of the Paris Agreement specifically mention agriculture [(Food and Agriculture Organization of the United Nations (FAO), 2019a)], highlighting its important role in mitigating GHG emissions. Additionally, 54 countries have set goals of decreasing emissions from livestock (Richards et al., 2015). Consequently, beef production, which has the greatest GHG emissions per gram of protein produced (Poore and Nemecek, 2018), is under increasing pressure to decrease emissions. The GHG intensity (kg CO_2 equivalent/kg carcass) of beef production continues to decrease over time in many countries (e.g., 14.5% decrease between 1981 and 2011 in Canada; Legesse et al., 2016), due to improvements in management, health, nutrition, and genetics of animals, as well as manure management, grazing management, crop production and decreased land conversion (Mayberry et al., 2019). However, a decrease in GHG intensity due to improved efficiency of production will not be sufficient to meet targets for absolute GHG reductions if animal production continues to expand to meet the demand for food security. As enteric CH_4 represents more than 50% of farm-based GHG emissions of beef production (Beauchemin and McGeough, 2013), reducing enteric CH_4 emissions has been identified as a key means of reducing emissions from the red meat sector (Mayberry et al., 2019). Thus, providing beef producers with effective mitigation options is critical. The present study conducted under commercial feedlot conditions confirms previous small scale research studies that show 3-NOP has tremendous potential for CH_4 mitigation for beef production (Vyas et al., 2016a, 2018).

Achieving carbon neutral beef production will undoubtedly increase the cost of production as well as the retail price of meat (Mayberry et al., 2019). Some of the additional costs to farmers of using CH_4 inhibiting feed additives may be at least partially offset by revenues from participating in voluntary carbon offset markets. For example, beef feedlots in Alberta, Canada, can participate in the Alberta Emission Offset System (www.alberta.ca/alberta-emission-offset-system.aspx) by quantifying reductions in CH_4 using scientifically valid methodologies. Furthermore, improvements in animal performance would lead to greater revenues per animal sold. The observed tendency in feed conversion efficiency improvement (2.5%) in the present study would be economically significant to the cattle industry, as feed costs represent the largest source of total input costs (>60%). Feed conversion efficiency has a substantial impact on revenue per animal sold (Retallick et al., 2013). For example, using current feed costs, a 1% improvement in feed conversion efficiency is estimated to save the Canadian feedlot sector $11.1 million annually (Buchanan-Smith and Wood, 2019). The Canadian beef industry is the 11th largest beef-producing country and the 5th largest exporter of beef globally [(Food and Agriculture Organization of the United Nations (FAO), 2019b)] with over 2.5 million cattle finished annually (Statistics Canada, 2019). In the U.S., where there are over 11 million beef cattle on feed at any one time (Cowley et al., 2019), the impact of feeding 3-NOP on reducing CH_4 emissions and improving feed conversion efficiency could be substantial. Furthermore, improvements in feed conversion efficiency decrease the demand for feed inputs resulting in fewer GHG emissions from feed production, less land required for feed production, and decreased manure output (Beauchemin et al., 2011). Thus, the observed 2.5% improvement in G:F and 21.7% decrease in CH_4 yield in the present study could have both significant environmental and economic implications for beef production systems in North America and elsewhere, if 3-NOP is approved by licensing authorities and made commercially available. However, further studies are needed to validate the efficacy of 3-NOP for CH_4 mitigation and determine its effects on animal performance when used in commercial beef production systems with varying animal types, diets (including high-grain finishing diets), and management conditions.

CONCLUSIONS

This research is the first to show the effects of feeding 3-NOP on feed consumption, animal performance, animal health and enteric CH_4 production of beef cattle fed a backgrounding diet in a commercial feedlot. Feeding 3-NOP tended to reduce DMI but improved G:F by 2.5%. No negative impacts on animal health (mortality and morbidity) were observed. Feeding 3-NOP resulted in a sustained reduction in enteric CH_4 yield of 22%, on average (ranging from 20 to 26% depending upon dose). Assuming it becomes commercially available, 3-NOP has great potential to reduce GHG emissions from the beef industry, particularly the feedlot sector where use of feed additives and nutritional supplements is commonplace.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Care committee at the Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada according to Canadian Council on Animal Care (2009) and with approval of the Veterinary Drug Directorate of Health Canada (DSTS No. 207171).

AUTHOR CONTRIBUTIONS

All authors have made a substantial direct and intellectual contribution to the work ranging from inception of the project idea, searching for funding, project implementation and management, data collection and analysis to writing, and editing of the manuscript. All authors have approved the manuscript for publication.

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The Importance of Farm Animal Health and Natural Behaviors to Livestock Farmers: Findings From a Factorial Survey Using Vignettes

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There is increasing interest in enabling positive experiences, not just minimizing negative experiences, to improve the welfare of farmed animals. This has influenced the growth of private agri-food standards and supported arguments to integrate animal welfare into policy on sustainability and climate change. However, much research finds that farmers predominantly focus on the minimization of negatives (i.e., health issues). This may impact the positioning of farmers within these wider societal debates, affecting their social license to farm. It is thus important to better understand farmers' priorities relating to the minimization of negative factors (e.g., health issues) and the promotion of positive experiences (i.e., natural behaviors). A novel 2 × 2 factorial survey using vignettes, which experimentally manipulated health (health issues minimized/not minimized) and natural behavior (natural behaviors promoted/not promoted) provision, was completed by livestock farmers ($n = 169$), mostly with extensive systems, in the UK and Republic of Ireland. The majority (88%) considered "minimizing health issues" to be the most important factor for animal well-being. However, the overall welfare of animals was judged to be highest when both health and natural behaviors were supported. Several individual characteristics, including farming sector, production system, gender, belief in animal mind and business type influenced how participants judged the welfare of animals and the level of importance they gave to health and natural behaviors. Findings suggest that although farmers prioritize the minimization of health issues they want animals to be both healthy and able to express natural behaviors, and individual characteristics are important for understanding farmers' welfare-related judgements.

Keywords: farm animal welfare, farmer attitude, sustainability, food policy, factorial survey analysis

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INTRODUCTION

There is growing awareness of and interest in the welfare benefits of promoting positive experiences in farm animals' lives, both within science and society. From a welfare science perspective, this is seen in the continued development of positive animal welfare, which emerged as a response to criticisms of an over-emphasis on minimizing negative aspects of welfare in traditional welfare science (Lawrence et al., 2019). From a societal perspective, it is evident in both the increasing expectations of members of the public and growing market-based presence of private agri-food standards. Much research finds that members of the public associate animal welfare with animals being able to experience positive aspects of life (Miele, 2010; Miele et al., 2011) and largely prioritize "naturalness" over other aspects of welfare (Spooner et al., 2014a; Cornish et al., 2016; Thorslund et al., 2016). In response, private agri-food standards and welfare schemes have increasingly sought to include assessment criteria considered indicative of natural behavior expression (e.g., days spent at pasture, outdoor access) (Lundmark et al., 2018; Vogeler, 2019). This has contributed to an emerging gap between public standards—which provide a minimum standard of welfare—and private agri-food standards which seek to meet public expectations for higher welfare (Henson and Reardon, 2005; Lundmark et al., 2018). This adds to the complexity of farm animal welfare policy, with private standards becoming increasingly dominant governance instruments in the market-driven context of farm animal welfare (Lundmark et al., 2018). There is also more recent evidence of both science and societal expectations for higher welfare informing debates of the importance of integrating animal welfare in wider policy issues, such as sustainability and food security (Buller et al., 2018), human health (European Commission, 2020), and climate change (Shields and Orme-Evans, 2015). Evidently, there is an awareness of a public desire for farm animals to experience positive lives (e.g., opportunities to express natural behaviors) and this is impacting both the development of and expectations for agri-food standards and related policy.

Yet, what of the farmers' position within these developments within science and society? Research finds that livestock farmers predominantly associate animal welfare with health and place particular emphasis on the minimization of pain, stress, disease, and other factors which may negatively impact the health of their animals (Bourlakis et al., 2007; Kauppinen et al., 2010; Faucitano et al., 2017). They also frequently link health and welfare to economic performance (Bourlakis et al., 2007; Skarstad et al., 2007; Kauppinen et al., 2010), using the productivity of their animals as an indicator of animal health and, in parallel, animal welfare (Bourlakis et al., 2007; Kauppinen et al., 2010; Vigors, 2019). Farmers' perspectives thus appear to be more closely aligned with the traditional welfare science views of minimizing harms as opposed to maximizing positive experiences. This may have implications for the position of farmers in respect to the growing interest in positive aspects of welfare seen in science (e.g., in positive animal welfare) and society (e.g., for "naturalness").

Although there is much evidence to suggest that farmers' attitudes to welfare are unidimensionally based around health (Hansson and Johan Lagerkvist, 2012), there is also evidence of heterogeneity in how farmers' construct and perceive animal welfare (Kirchner et al., 2014). Importantly, one of the main variances noted is in the importance farmers give to natural behaviors, where such variances have been linked to differences between farmers in welfare scheme (Kjaernes et al., 2008), production system (Spooner et al., 2014b), organic status (Skarstad et al., 2007), values (Hansson et al., 2018), and attitudes to animals (Hanna et al., 2009). As such, when variances between and within individual farmers are considered, welfare-related attitudes and decision-making may be more complex than is often suggested. This becomes important within a wider societal context where the views of farmers and the public are often presented as being discordant, with studies highlighting the health focus of farmers on the one side and the "naturalness" focus of the public on the other (Te Velde et al., 2002; Vanhonacker et al., 2008; Cornish et al., 2016). Such views have led to concerns that farmers may be at risk of failing to meet public demand and expectations relating to positive aspects of welfare (e.g., natural behaviors) (Hansson and Johan Lagerkvist, 2012). Yet, if there is heterogeneity between farmers in terms of their attitudes to welfare, it may only be particular groups or types of farmers who may be at risk in this respect.

At the same time, farmers have become the focus of public policy debates which, in seeking to address climate change, sustainability, and food security, may be at odds with public expectations for greater positive welfare and the values of some farmers (as above) relating to natural behaviors. Namely, "sustainable intensification," whereby farm animal production is encouraged to be more efficient (i.e., produce more with less resources), has been proposed as a means to reduce emissions from animal production, whilst supporting food security and sustainability (Garnett et al., 2013; Shields and Orme-Evans, 2015). However, research within welfare science has demonstrated that increasing the efficiency of animal production can come at the cost of animal's engaging in positive experiences (e.g., natural behaviors) (Rayner et al., 2020). Farmers are thus faced with operating within in an increasingly complex environment where protecting their 'social license' to farm could depend on how they address expectations for higher welfare along with climate change and sustainability issues (Williams and Martin, 2012). As Buller et al. (2018, p. 5) explain "The challenges facing agriculture over the next half-century are formidable; to be less environmentally damaging yet significantly increase food production while maintaining acceptable levels of animal welfare and human health."

Given the complex backdrop of increasing scientific and societal interest in supporting positive aspects of animals lives (e.g., natural behaviors) and the potential discordance between public expectations for welfare, the "sustainable intensification" of farming and farmers' welfare-related perspectives, it is important to better understand how farmers' prioritize key elements of welfare (i.e., minimizing health issues, promoting natural behaviors). Arguably, key policy decisions will have to be made in this context in the near future; having a better understanding of farmers' perspectives on key elements of welfare is therefore important for both designing policies on farm animal welfare (e.g., governance of welfare) and effectively situating welfare into wider policy debates (e.g., sustainability). As such, this study has two specific aims. First, it seeks to determine how important minimizing health issues and promoting natural behaviors are within farmers' welfare-related attitudes and judgements. Secondly, it aims to explore the extent to which individual differences (e.g., in sector, production system) influence farmers' attitudes to the importance of health and natural behaviors and their welfare-related judgements.

RELEVANT LITERATURE

In the context of animal welfare, livestock farmers are primarily found to focus on and prioritize the minimization of health issues (Cornish et al., 2016). This is a consistent feature of farmers' perspectives on welfare, often regardless of sector, management system, or individual values and beliefs (Te Velde et al., 2002; Hansson and Johan Lagerkvist, 2012; Spooner et al., 2012; Hansson et al., 2018). However, beyond health, "a real diversity exists…among farmers when considering [animal welfare]" (Dockès and Kling-Eveillard, 2006, p. 248).

One of the main differences noted is in farmers' attitudes to the importance of or role of natural behaviors in animal welfare. Here, much research links differences to variances in individual characteristics between farmers. For instance, Spooner et al. (2014b) found that producers who kept group-housed pigs emphasized natural behaviors more than producers who did not keep group-housed pigs; suggesting their welfare perspectives may have been influenced by the management and production system they operate or were familiar with. Farmers participating in organic or welfare-specific assurance schemes have also been found to place greater emphasis on natural behaviors than those in standard schemes (Bock and van Huik, 2007) and the type of business farmers operate (e.g., conventional, organic, large enterprises) may underlie their intrinsic (e.g., a personal desire) or extrinsic (e.g., to remain competitive) motivations to participate in welfare schemes (Bourlakis et al., 2007). Similarly, Skarstad et al. (2007) found that non-organic farmers referred to welfare in terms of good animal health while organic farmers associated welfare with natural behaviors. Interestingly, Dockès and Kling-Eveillard (2006) found a link between the type of production system a farmer had and their values; individuals with systems which support natural behaviors (e.g., group-housed calves, pigs on straw) described how they chose these systems because they were in line with their ethical values. The work of Hansson et al. (2018) further reveals how differences in personal values can lead to differences between farmers in terms of their attitudes to welfare. They found that "animal-centered" farmers placed more emphasis on non-use values (e.g., animal well-being) of animal welfare than farmers with "human-centered" or "business-oriented" values. Level of empathy and an individuals' attitude to animals are also known to impact farmers' assessments of and approach to managing welfare, with positive attitudes and higher levels of empathy associated with better welfare outcomes (Hanna et al., 2009; Kielland et al., 2010). Thus, when viewed together, attitudes to the importance of natural behaviors may vary between individual farmers, influenced by differences in management and production systems, sector, organic status, personal values, and attitude to animals.

More specific to positive welfare, farmers have been found to emphasize husbandry practices which may promote positive welfare and support natural behavior expression, such as; enabling animal autonomy, supporting play and social interactions, encouraging positive affect and developing positive human-animal relationships (Vigors and Lawrence, 2019). Notably, the priority farmers' gave to these different aspects varied according to their sector, management system, personal values, and the specific context of their farm and animals (Vigors and Lawrence, 2019). For instance, farmers who were found to value a more loosely-structured management system (i.e., use of limited human intervention) emphasized the importance of giving animals opportunity for autonomy over other welfare provisions. Conversely, this was less of a priority for farmers who valued a highly-structured management system (i.e., close control and monitoring of animals) (Vigors and Lawrence, 2019). Differences in the role farmers perceived they had in promoting natural behaviors, such as play, were also evident between different sectors. Those with more intensive systems, such as pig and poultry, described providing objects to support play or provide environment enrichment (Vigors and Lawrence, 2019). Conversely, farmers from sectors with extensive systems, such as beef and sheep, felt no direct promotion of play or other natural behaviors were required; this was perceived to be supported by the "natural," outdoor environment innate to their management system (Vigors and Lawrence, 2019). Thus, differences in values, sector and management system, again, may account for differences between farmers in terms of their attitudes toward and emphasis of natural behaviors.

In sum, although it is clearly evident that farmers strongly emphasize and prioritize the minimization of health issues within their management practices and welfare-related decision-making, there is also evidence that they consider the importance of natural behaviors to animal welfare. However, the importance farmers place on natural behaviors may be influenced by individual characteristics and differences (e.g., in values, management system, sector) resulting in greater heterogeneity in attitudes to natural behaviors than in attitudes to health. These warrant deeper investigation, particularly in the context of the, previously discussed, complex social and policy environment farmers operate in.

In response, this study seeks to examine how important farmers consider health and natural behaviors to be and under what conditions, whilst also exploring the extent to which personal characteristics may account for any differences in attitudes toward and judgements of animal welfare. Using a novel factorial survey design, we examine how varying levels of health and natural behavior provision influence farmers' judgements and assessments of different attributes of animal welfare. In addition, we directly examine how farmer characteristics (e.g., management system, assurance scheme membership, organic, sector etc.) may influence the importance they place on health and natural behaviors and how they judge the welfare of animals.

MATERIALS AND METHODS

Survey Design

Real-world judgement and decision-making often involves the consideration of multiple, complex, factors which have to be weighed against each other in order to reach a judgement (Taylor, 2006). This is certainly the case with animal welfare, where trade-offs frequently have to be made between different welfare provisions and decisions made based upon the specific nature of the situation or context (Appleby et al., 2014; Sandøe et al., 2019). Moreover, judgements are often influenced by the personal characteristics of the individual (Hox et al., 1991), as evident in the previous section.

Factorial surveys using vignettes provide a method to examine such complex judgements, where both the particulars of the situation in question and the individual's personal characteristics can impact judgement and decision-making (Hox et al., 1991). In a factorial survey, participants are asked to make judgements along specified dimensions based on information provided to them in a vignette (Hox et al., 1991). A vignette is a short description of a scenario created from the systematic selection and experimental manipulation of factors under study (Hox et al., 1991; Atzmüller and Steiner, 2010). By combining such experimental approaches with a traditional survey format (i.e., collect data on respondent-specific characteristics), factorial surveys offer a powerful means to determine which vignette factors may causally affect individual judgements and the underlying influence of personal characteristics on judgements (Hox et al., 1991; Taylor, 2006).

A factorial survey approach was taken in this study to examine how the two key factors in question —the importance of minimizing health issues and promoting natural behaviors—impacted farmers' judgements and assessments of welfare and the extent to which personal characteristics influenced variances in judgements between participants. This approach resulted in the survey containing several key sections: (i) a factorial vignette scenario to capture participants' judgements of animal welfare under varying levels of health and natural behavior provision; (ii) measures of participant characteristics and demographic factors; (iii) attitudinal measures to capture overall attitude to the importance of health and natural behaviors. These sections are described in further detail below.

The survey (see **Supplementary Material**) was approved by Scotland's Rural College Social Science Ethics Committee and by the Scottish Government's Rural Affairs Food and the Environment Strategic Research programme.

TABLE 1 | Vignette scenarios.

Farm 1: High Health × Low Behavior	Farm 2: High Health × High Behavior
"I want my animals to be healthy. To me, this means having them stress free, pain free and injury free, whilst also being aware of any health issues that might be arising and dealing with them. At the same time, I don't think I need to do anything specific to support natural behavioral expression in my animals"	"I want my animals to be healthy. To me, this means having them stress free, pain free and injury free, whilst also being aware of any health issues that might be arising and dealing with them. At the same time, I want my animals to be able to express their natural behaviors. So, I try to make sure that they can go and have a wander around and see their surroundings, they can choose the animals they want to be around, lie down where they want to lie down and eat when they want to eat"
Farm 3: Low Health × Low Behavior	**Farm 4: Low Health × High Behavior**
"When it comes to health, I am inclined to let nature take its course. I'd rather let the animal look after itself than intervene. For example, If I see the odd animal with a sore foot, I'll leave it alone and let it heal in its own time. At the same time, I don't think I need to do anything specific to support natural behavioral expression in my animals"	"When it comes to health, I am inclined to let nature take its course. I'd rather let the animal look after itself than intervene. For example, If I see the odd animal with a sore foot, I'll leave it alone and let it heal in its own time. At the same time, I want my animals to be able to express their natural behaviors. So, I try to make sure that they can go and have a wander around and see their surroundings, they can choose the animals they want to be around, lie down where they want to lie down and eat when they want to eat"

Judgement of Animal Welfare: 2 × 2 Factorial Vignette

A factorial vignette, using a 2 × 2 experimental design, formed the main part of the survey. Participants were presented with one singular vignette; a hypothetical scenario describing the approach of a livestock farmer to minimizing health issues and promoting natural behaviors on their farm. Vignettes were created by manipulating the two factors of central interest in this study; health provision and natural behavior provision, and their two levels; health issues not minimized/health issues minimized and natural behaviors promoted/natural behaviors not promoted. This resulted in four possible vignettes, as described in **Table 1**. It is important to note that participants did not see the labels (e.g., farm 1: high health × low behavior) of each vignette, only the vignette narrative. In addition (see **Supplementary Material**), a descriptor of what is meant by "health" and "natural behaviors" was included below the vignette narrative.

Going forward, health issues minimized will be abbreviated to HH (High Health), health issues not minimized to LH (Low Health), natural behaviors promoted to HB (High Behavior), and natural behaviors not promoted to LB (Low Behavior). The wording and phrases used in the vignettes were taken from livestock farmers' descriptions of how they manage the health and promote the natural behaviors of their animals, collected during a prior qualitative interview study completed by the authors (see Vigors and Lawrence, 2019). This was done to ensure the vignettes reflected real-world conditions, used language relevant to livestock farmers and harnessed the validity of "folk" rather than scientific definitions of welfare (Weary and Robbins, 2019). In addition, the vignettes were framed in terms of the behavior or actions of a hypothetical farmer in proactively seeking to minimize (or not) health issues or directly promote (or not) natural behaviors rather than animals being e.g., healthy *per se.*

The four vignettes were randomized so that each respondent received only one vignette scenario. This is a recommended approach for factorial surveys to reduce the potential for response fatigue (caused by multiple vignette sets) and to ensure independence of observations between individuals, whilst also adding to the robustness of the experimental design (Taylor, 2006). Based on the information provided in the vignette, participants were then asked to rate (i.e., judge) (on a slider scale from 0 to 10, where 0 indicated poor, 5 average, and 10 excellent) several dimensions relevant to animal welfare: (i) the overall well-being of the animals in the scenario; (ii) the physical health of the animals; (iii) the mental health of the animals and; (iv) the productivity of the animals. The latter was considered important for inclusion in light of the previously discussed literature which suggests farmers link welfare and health with the productivity of their animals (e.g., Bourlakis et al., 2007; Skarstad et al., 2007; Kauppinen et al., 2010). The purpose of this section was to examine the impact of varying, and at times conflicting, levels in provisions for health and natural behaviors on respondents' assessments of different welfare attributes—animal well-being, physical and mental health, and productivity. This enables an examination of how the rating of welfare attributes may causally vary according to variances in the level of health and natural behavior provision (i.e., high health/low health, high behavior, low behavior).

The vignette section of the survey also included a question aimed at capturing how respondents thought other farmers would rate the overall well-being of the animals described in the vignette. This was included to examine the potential effect of social norms, whereby participants may respond in a manner they think is in line with the expectations of others (i.e., peers). The design of this question followed the recommendations of Bicchieri (2016), who describes that one way to assess social norms is to ask people how they believe others may respond to a similar question.

Two open-ended qualitative questions were also included in the vignette section. Here, participants were asked to (i) explain why they gave the overall well-being rating that they did, and (ii) describe what, if anything, they would change about the farm described in the vignette. This was done to provide richer detail on participants' reasoning and perceptions of the vignette scenario.

Participant Characteristics

The survey collected relevant socio-demographic and farmer characteristics. This is a key element of factorial surveys, which combine experimental design (i.e., 2 × 2 factorial vignettes) with traditional survey design, enabling investigation at the vignette and individual level and how both impact judgements and attitudes (Hox et al., 1991; Taylor, 2006). Socio-demographic measures included multiple-choice questions for gender, age, highest level of education, annual household income, dietary preferences (i.e., regularly eat meat, flexitarian, vegetarian, vegan, pescatarian, other), type of area living (i.e., urban, rural), and geographic location (i.e., UK, Republic of Ireland). The belief in animal mind (BAM) scale (from Knight et al., 2004) was also included to capture respondents' attitudes to animal sentience. The scale included four questions, with participants rating the extent to which they agreed (on a slider scale from 0 to 10, with 0 indicating complete disagreement and 10 complete agreement) that farm animals: (i) are unaware of what is happening to them (i.e., not conscious); (ii) capable of experiencing feeling and emotion; (iii) able to think to some extent to solve problems and make decisions; and are (iv) like computer programs, responding to urges without awareness of what they are doing (Hills, 1995; Knight et al., 2004).

Questions relevant to individual characteristics thought to impact farmers' perspectives of welfare were also included. Specifically, multiple-choice questions for organic status (organic, non-organic), membership in farm assurance scheme (whether member of a scheme or not), farming sector (sheep, beef, dairy, pig, broiler chicken, laying-hen, poultry other, other) management system (e.g., whether animals were housed year-round or part of the year, etc.), type of farm business (family-run, commercial partnership, direct-to-buyer, small-holding, other), tenure (number of years farming), and the number of animals they manage. A self-reported measure of input intensity—the extent to which inputs such as concentrated feeds and fertilizers are increased to produce one unit of output (European Commission, 2017)—was also included to gain a general insight into the intensiveness/extensiveness of each respondent's farm.

Overall Attitude to Importance of Minimizing Health Issues and Promoting Natural Behaviors

A further section (separate from the vignette section and presented at a later stage in the survey) captured respondents' overall attitude to the importance of minimizing health issues and promoting natural behaviors within farm animal welfare. Participants were asked to rate (on a scale from 0 to 10, where 0 indicated not important at all, 5 of average importance, and 10 extremely important), how important they considered (i) the minimization of health issues and (ii) the promotion of natural behaviors to be for the overall well-being of farm animals. To further determine which factor participants considered was the most important, a binary choice question asked respondents to select between "minimizing health issues" and "promoting natural behaviors" as the most important factor for animal well-being.

Data Collection

The survey was open to livestock farmers, from all sectors, and of all farm sizes, in the UK and Republic of Ireland. The survey was disseminated online using social media and in online newsletters with the assistance of several farming sector organizations, agricultural colleges and the farming press. As such, a non-probability sampling approach was used. No incentives for completing the survey were offered, with participants completing the survey on a voluntary basis.

Data Preparation

The survey received 248 responses. Responses that were mostly incomplete (e.g., more than half of survey not completed) were removed, resulting in a final sample of 169 individuals (which contained a small number of incomplete responses for some participant characteristic categories) and a 68% item non-response rate. Quantitative responses were entered into SPSS, Version 25 (IBM Corp, 2017), for analysis. Data was checked for multicollinearity and normality. There were no multicollinearity issues (as assessed by a VIF <10) within the explanatory variables (i.e., participant characteristics and level of health and behavior provision in vignette scenario). Normality was also assessed by a visual inspection of Q-Q Plots and the distributions were determined as normal.

Several categories of the participant characteristic variables were removed or recategorised for quantitative analysis due to some being unselected or having small sample sizes. Gender was regrouped to "male" and "female" as the "in another way" and "prefer not to say" categories had no responses. Education was regrouped into "non-degree" (combining "primary" and "secondary"), "undergraduate," "post-graduate," and "other." For dietary preferences, "vegan" was removed (due to no responses), vegetarian and pescatarian was regrouped into a "does not eat meat" category, "regularly eat meat" responses were recategorised as "eats meat" and the flexitarian category remained unchanged. The type of area living variable was recategorised as either urban (combining "urban" and "suburban") or rural (combining "semi-rural" and "rural"). The "broiler chicken" category was removed from the farming sector variable, as it was unselected. Responses relating to "sector" and "type of farm enterprise" were multiple response; participants could select more than one category (e.g., beef and sheep). Each category within both these variables were transformed into dummy variables (e.g., member of beef sector or not member of beef sector) and treated as singular predictor variables. The four item BAM scale was examined for reliability and found to have a Cronbach's alpha of 0.60. This is in line with previous applications of this scale (i.e., Knight et al., 2004 reported a Cronbach's alpha of 0.62). A summated scale of BAM was thus created to provide a mean score for each participant's overall BAM (i.e., higher means indicated a greater belief in farm animal mind).

Data Analysis

Analysis of Factorial Vignettes

There were high levels of correlation (Cronbach's alpha >0.7) between the vignette scenario outcome variables (i.e., ratings of well-being, physical and mental health, productivity, and social norms). As such, a multivariate linear regression was used to examine the impact of the different levels of health (i.e., HH/LH) and natural behavior (i.e., HB/LB) provision, and participant characteristics on the vignette judgement variables (i.e., well-being, physical health, mental health, social norms). To assess the overall model, only terms statistically significant at the 5% level were included in the model and its overall performance was measured by the adjusted η^2 of value of the model (adjusted η^2 gives the proportion of variance explained adjusted by the number of terms in the model). Following this, all statistically significant and non-significant terms were included and the model refitted to examine the full range of the effects of the explanatory variables (i.e., health and natural behavior provision, participant characteristics) on the outcome variables (i.e., well-being, physical health, mental health, productivity, social norms). Wilks' λ (which considers each term having been adjusted for inclusion of the others) was used to assess the impact of the explanatory variables on the combined outcome variables. Assessments of partial η^2 were used to determine the individual effects of each explanatory variable on each single outcome variable. To examine the impact of social norms, a paired *t*-test was used to assess the difference between how participants rated overall well-being and the rating they gave for how they thought other farmers would rate well-being.

Analysis of Qualitative Responses to Vignette Scenarios

Qualitative responses to the vignette scenario questions; (i) Why did you give this rating for overall well-being? and; (ii) If you were to change anything about this farm; what would that be? were entered into MaxQDA (Version 2018.2) for analysis. Responses to (i) were first analyzed using a sentiment analysis approach, which involved categorizing responses according to whether they were positive, negative, or neutral in terms of how the participants felt about the vignette scenario they were assigned to. Responses within each vignette and within each sentiment category were then further analyzed thematically. This involved working through each response and coding their content according to the specific points, or themes, discussed by respondents. This resulted in several overarching themes within each sentiment category (i.e., positive, negative, neutral), and sub-themes capturing reasons why participants rated well-being as they did. Responses to (ii) were analyzed using a content analysis approach. This involved looking for commonality in descriptive words conveying what participants would change and counting, using MaxQDA software, how often they were mentioned across participants.

Analysis of Overall Attitude to Importance of Minimizing Health Issues and Promoting Natural Behaviors

A paired samples *t*-test was used to determine differences in participants' ratings (on a scale of 0–10) of the importance of "minimizing health issues" and "promoting natural behaviors."

A cumulative odds ordinal logistic regression with proportional odds was run to determine the effect of participant characteristics (i.e., gender, age, education, income, tenure, dietary preferences, type of area living, geographic location, assurance scheme membership, farming sector, management system, business type, number of animals managed, organic status, BAM, input intensity) on the importance given to minimizing health issues and promoting natural behaviors. The vignette health and behavior provision variables were also included as predictors in the model to determine whether the information participants were previously exposed to in the vignette scenario had any effect on how they rated the importance of health and natural behaviors.

The following section presents the results of the aforementioned analyses. Throughout, when not otherwise stated, "significant" means significant at the 5% level.

RESULTS

Sample Descriptives

The majority of the sample (62%) were from the United Kingdom (UK). There was an almost even split between genders (53% male, 47% female) for the total sample (however, more females were from the UK). The mean age of the total sample was 41 (range: 18–75). The majority of the sample were educated to degree level, holding either undergraduate (44%) or postgraduate (22%) certifications. Participants with "other" levels of education (17%) included those with vocational training qualifications (e.g., NVQ's) and other third-level education such as higher diplomas. An annual household income of £20,000–£34,999 was the most common response (29%) amongst UK participants, while for Irish respondents a household income of £35,000–£49,999 was the most common (20%). Dietary preferences were somewhat homogeneous with a large majority (92%) indicating they regularly eat meat. Almost all participants (96%) lived in rural areas. The majority (31%) had been farming for more than 30 years, closely followed by those who had been farming between 11 and 20 years (27%).

The majority of participants were from the beef (54%) and/or sheep (52%) sector, with most Irish participants (63%) from the beef sector and most UK participants (70%) from the sheep sector. The dairy sector was the third most prevalent (27%), followed by a smaller number in the pig (9%), laying-hen (9%), and "other poultry" sectors (e.g., duck, turkey) (3%). A small number (8%) also selected "other" for sector which included responses such as keeping goats. Most participants had a family-run business (71%) and were a member of a farm assurance scheme (67%). There were very few participants who farmed organically (3%) and these were only in the UK. Beyond the majority of the sample who were non-organic (86%), 4% selected "other" describing various different approaches such as, "free range dairy," "farming with an organic ethos," and "limited use of chemicals." With regards to how they managed their animals, the majority of the sample (50%) indicated their animals were housed for part-of the year and outdoors for part-of the year, followed by those who indicated their animals were outdoor all-year round (21%). Only a small number (7%) housed animals all year-round, most of which were in the UK ($n = 10$) and 1 in the ROI. Several participants (11%) also selected "other" for management system, describing approaches such as animals being outdoors all year but with free access to a barn, only housing animals in extreme weather conditions, or species-specific management (e.g., "cattle housed in winter, sheep outdoors all the time" or "sheep housed only for lambing"). The remainder housed their animals but gave them access to the outdoors some of the time (2%), or all of the time (2%). The mean rating for BAM was 7.66 ($SD = 1.5$) and the mean rating for input intensity was 4.88 ($SD = 2.19$). Complete demographic and demographic information split by country (i.e., UK and ROI) can be found in **Table 2**.

Factorial Vignette Findings

The random assignment of participants in the different vignettes resulted in 24% being assigned to farm one (HH × LB), 21% to farm two (HH × HB), 28% to farm three (LH × LB), and 27% to farm four (LH × HB). To determine how participants judged the welfare of the animals within each scenario, the mean ratings for each welfare judgement attribute (i.e., ratings for overall well-being, physical health, mental health, and productivity) within each vignette were examined. Farm two (HH × HB) received the highest mean ratings for each welfare attribute, followed by farm one (HH × LB) and farm four (LH × HB). Farm three (LH × LB) received the lowest mean ratings for all welfare judgement variables. **Figure 1** displays the difference in mean judgement ratings for each welfare attribute across the different levels of health (i.e., high health/low health) and behavior provision (i.e., high behavior/low behavior).

Pairwise comparisons of mean differences indicated that the differences in judgements of welfare attributes between the different vignette scenarios were statistically significant. **Table 3** presents these significant mean differences and ranks each vignette scenario according to the highest and lowest mean rating for each judgement variable (i.e., well-being, physical health, mental health, productivity, social norms). Of particular note are the mean differences between scenarios where there is some trade-off in the provision of health and natural behaviors i.e., farm 1 (HH × LB) and farm 4 (LH × HB). As indicated by **Figure 1** and **Table 3**, a scenario where health provision is high but behavior provision is low (i.e., farm 1) results in higher mean ratings for each judgement variable than scenarios where health provision is low but behavior provision is high (i.e., farm 4). In other words, low health provision results in low mean ratings of welfare attributes even if behavior provision is high. A slight exception to this is for ratings of mental health, where the mean difference between farm 1 and farm 4 is slight ($M = 0.31$, $p < 0.01$; see **Table 3**), although still in favor of high health provision resulting in higher mean ratings than high behavior, as evident in **Figure 1** above.

To capture social norms, participants were also asked to rate how they thought other livestock farmers would rate the overall well-being of the animals described in the vignette they were assigned to. Paired samples t-tests revealed that respondents assigned to farm one (HH × LB), farm three (LH × LB), and farm four (LH × HB) vignettes believed that other livestock farmers would give higher ratings for well-being than them, with significant respective mean differences of .73 (95% CI, 0.21–1.24, $p = 0.007$), 0.88 (95% CI, 0.35–1.40, $p = 0.002$), and 0.67 (95% CI, 0.12–1.22, $p = 0.017$). There was no evidence that participants who received the farm two (HH × HB) vignette believed that other livestock farmers would rate the overall well-being of the animals in the scenario any differently than they did, with an estimated mean difference of −0.37 (95% CI, −0.87–0.12, $p = 0.135$).

The Impact of Vignette Factors and Participant Characteristics on Judgements of Vignette Scenarios

A multivariate regression was fitted to examine the impact of the different levels of the vignette conditions and participant characteristics on judgement variables (i.e., well-being, physical health, mental health, social norms). In the model including only statistically significant explanatory variables the combined predictor variables (i.e., health provision, behavior provision, participant characteristics) had the greatest effect on judgements of overall well-being, $F_{(57,154)} = 4.58$; $p < 0.001$; adjusted $\eta^2 = 0.60$ (where adjusted η^2 gives the proportion of variance explained adjusted by the number of terms in the model). This was followed by physical health, $F_{(57,154)} = 3.72$; $p < 0.001$; adjusted $\eta^2 = 0.52$, productivity, $F_{(57,154)} = 3.42$; $p < 0.001$; adjusted $\eta^2 = 0.51$, social norms, $F_{(57,154)} = 3.18$; $p < 0.001$; adjusted $\eta^2 = 0.50$ and mental health judgements, $F_{(57,154)} = 2.56$; $p < 0.001$; adjusted $\eta^2 = 0.40$.

TABLE 2 | Demographic data of study participants.

	UK (*n* = 105)		Ireland (*n* = 64)		Total (*n* = 169)	
	Number	%	Number	%	Number	%
Gender						
Male	40	38	49	77	89	53
Female	65	62	15	23	80	47
Age						
18–29	13	12	13	20	26	15
30–39	15	14	18	28	33	20
40–49	21	20	8	13	29	17
50–59	17	16	11	17	28	17
60 and over	27	26	8	13	35	21
Prefer not to say	12	11	6	9	18	11
Education						
Non-degree	19	18	10	16	29	17
Undergraduate Degree	45	43	30	47	75	44
Post-graduate Degree	21	20	16	25	37	22
Other	20	19	8	13	28	17
Household income						
<20,000	13	12	5	8	18	11
20,000–34,999	30	29	11	17	41	24
35,000 to 49,999	24	23	13	20	37	22
50,000–74,999	11	11	13	20	24	14
75,000–99,999	7	7	7	11	14	8
Over 100,000	5	5	5	8	10	6
Prefer not to say	15	14	10	16	25	15
Dietary preferences						
Meat is regular part of diet	94	90	62	97	156	92
Flexitarian	5	5	0	0	5	3
Does not eat meat	6	6	2	3	8	5
Type of area living						
Non-Rural	1	1	5	8	6	4
Rural	104	99	59	92	163	96
Number of years farming						
5 years and under	10	10	5	9	15	10
6–10	12	12	10	18	22	14
11–20	26	26	16	28	42	27
21–30	19	19	9	16	28	18
More than 30	32	32	16	28	48	31
Prefer not to say	1	1	1	2	2	1
(Missing due to incomplete data)					12	7
Sector						
Sheep	70	70	12	21	82	52
Beef	48	48	36	63	84	54
Dairy	11	11	31	54	42	27
Pig	12	12	2	4	14	9
Laying-hen	12	12	2	4	14	9
Poultry other	4	4	0	0	4	3
Other	12	12	1	2	13	8
Number of animals managed						
Unspecified	1	1	1	2	2	1
<99	21	21	13	23	34	22

(Continued)

TABLE 2 | Continued

	UK ($n = 105$)		Ireland ($n = 64$)		Total ($n = 169$)	
	Number	**%**	**Number**	**%**	**Number**	**%**
100–299	32	32	26	46	58	37
300–499	11	11	12	21	23	15
500–999	13	13	3	5	16	10
1,000–4,999	19	19	1	2	20	13
5,000–9,999	2	2	1	2	3	2
More than 10,000	1	1	0	0	1	1
(Missing due to incomplete data)					12	7
Type of farming enterprise						
Family-run	70	70	50	88	120	71
Commercial partnership	4	4	3	5	7	4
Direct-to-buyer (e.g., farm-shop)	2	2	0	0	2	1
Small-holding	26	26	5	9	31	18
Other	3	3	0	0	3	2
(Missing due to incomplete data)					6	4
Assurance scheme member						
Yes	63	63	50	88	113	67
No	34	34	4	7	38	23
Prefer not to say	3	3	3	5	6	4
(Missing due to incomplete data)					12	7
Organic						
Organic	5	5	0	0	5	3
Non-organic	89	89	56	98	145	86
Other	6	6	1	2	7	4
(Missing due to incomplete data)					12	7
Management system						
Animals housed all year-round	10	10	1	2	11	7
Animals housed part-of-year and outdoor part-of-year	35	35	50	88	85	50
Animals housed but free access to outdoors all of the time	3	3	0	0	3	2
Animals housed but free access to outdoors some of the time	4	4	0	0	4	2
Animals outdoor all year-round	31	31	5	9	36	21
Other	17	17	1	2	18	11
(Missing due to incomplete data)					12	7

Percentages rounded to whole number.

In the model including all the explanatory variables, including participant characteristics and health and behavior provision, information on whether health issues were minimized or not (i.e., health provision variable) significantly explained more of the variability in the combined judgement variables (i.e., well-being, physical health, mental health, productivity, social norms) than any other predictor variable, Wilks' $\Lambda = 0.394$; $F_{(5,93)} = 28.62$; $p < 0.001$. The vignette information participants received on whether natural behaviors were promoted or not (i.e., behavior provision variable) also had a significant impact on the combined

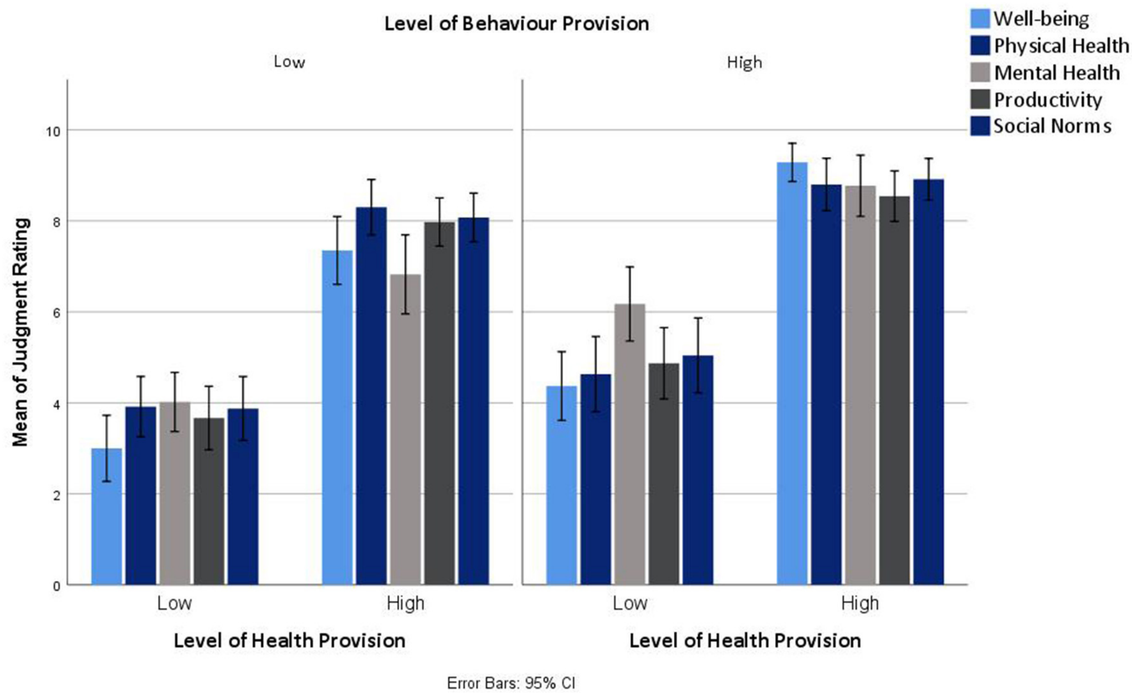

FIGURE 1 | Mean rating of well-being, physical health, mental health, productivity, and social norms by levels of health and natural behavior provision.

judgement variables, although it explained less of the variance than health provision; Wilks' $\Lambda = 0.71$; $F_{(5,93)} = 7.54$; $p < 0.001$. None of the participant characteristic variables included in the multivariate model significantly explained the variance of the combined outcome variables.

In terms of the impact of each predictor variable on each judgement variable (i.e., well-being, physical health, mental health, productivity, social norms), following adjustment for other variables, health provision, again, explained more of the variance of each judgement variable than any other predictor variable. Its greatest impact was on judgements of overall well-being; $F_{(1,97)} = 117.54$; $p < 0.001$; $\eta^2 = 0.55$, followed by productivity; $F_{(1,97)} = 99.80$; $p < 0.001$; $\eta^2 = 0.51$, physical health; $F_{(1,97)} = 99.40$; $p < 0.001$; $\eta^2 = 0.51$, social norms; $F_{(1,97)} = 92.09$; $p < 0.001$; $\eta^2 = 0.49$ and finally, mental health; $F_{(1,97)} = 34.25$; $p < 0.001$; $\eta^2 = 0.26$. Behavior provision also had a significant (but lower than health provision) effect on each of the judgement variables. Its strongest effect was on judgements of mental health; $F_{(1,97)} = 27.66$; $p < 0.001$; $\eta^2 = 0.22$, followed by well-being; $F_{(1,97)} = 27.01$; $p < 0.001$; $\eta^2 = 0.22$, social norms; $F_{(1,97)} = 14.06$; $p < 0.001$; $\eta^2 = 0.13$ and productivity; $F_{(1,97)} = 11.13$; $p = 0.001$; $\eta^2 = 0.10$. Its lowest effect was on ratings of physical health; $F_{(1,97)} = 8.45$; $p = 0.005$; $\eta^2 = 0.08$.

Several participant characteristic variables also had a significant effect on some of the judgement variables. As depicted in **Table 4**, operating either a family-run business or commercial partnership was a positive predictor of judgements of well-being, physical, and mental health. Having a family-run business was also a positive predictor of judgements of animal productivity. This indicates that participants with these business types judged the welfare of animals in each scenario to be more positive than those of other business types (i.e., direct-to-buyer, small-holding). Being a member of the beef sector also had a significant effect on judgements of well-being, physical health, and productivity. Specifically, beef sector was a positive predictor, indicating members of the beef sector judged these welfare attributes to be more positive than those from other farming sectors (i.e., sheep, dairy, pig, laying-hen, poultry-other, other). Dairy sector also had a significant, but negative, effect on judgements of mental health indicating that members of the dairy sector judged mental health to be lower than participants from other sectors. BAM also had a significant and positive effect on judgements of productivity and social norms, indicating that participants with a greater BAM rated the ability of an animal to be productive (in the different vignette scenarios) higher than those with a lower BAM. Similarly, participants with a greater BAM gave higher ratings for social norms than those with a lower BAM. The remaining participant characteristics, including geographic region (i.e., UK or ROI) were not found to have a significant effect on the judgement variables.

Qualitative Responses: What Influenced Participants Ratings of Overall Well-Being?

In order to gain a deeper insight into what may have influenced participants' judgements and how they decided on a rating for animal well-being, an open-ended question asked respondents to discuss why they gave the rating for overall well-being that they did. As previously described, these findings were analyzed both

TABLE 3 | Pairwise comparison of mean differences between vignette scenarios for judgements of welfare attributes.

	Reference category				Ranking
	Farm 1	**Farm 2**	**Farm 3**	**Farm 4**	
Well-being					
Farm 1 (HH × LB)	0	−2.65*	4.27*	2.48*	2nd
Farm 2 (HH × HB)	2.65*	0	6.92*	5.13*	1st
Farm 3 (LH × LB)	−4.27*	−6.92*	0	−1.79*	4th
Farm 4 (LH × HB)	−2.48*	−5.13*	1.79*	0	3rd
Physical health					
Farm 1 (HH × LB)	0	−1.03*	4.58*	3.10*	2nd
Farm 2 (HH × HB)	1.03*	0	5.61*	4.13*	1st
Farm 3 (LH × LB)	−4.58*	−5.61*	0	−1.48*	4th
Farm 4 (LH × HB)	−3.10*	4.13*	1.48*	0	3rd
Mental health					
Farm 1 (HH × LB)	0	−2.30*	2.87*	0.31*	2nd
Farm 2 (HH × HB)	2.30*	0	5.17*	2.61*	1st
Farm 3 (LH × LB)	−2.87*	−5.17*	0	−2.56*	4th
Farm 4 (LH × HB)	−0.31*	−2.61*	2.56*	0	3rd
Productivity					
Farm 1 (HH × LB)	0	−1.21*	4.5*	2.88*	2nd
Farm 2 (HH × HB)	1.21*	0	5.71*	4.08*	1st
Farm 3 (LH × LB)	−4.5*	5.71*	0	−1.62*	4th
Farm 4 (LH × HB)	−2.88*	−4.08*	1.62*	0	3rd
Social norms					
Farm 1 (HH × LB)	0	−1.60*	4.33*	2.63*	2nd
Farm 2 (HH × HB)	1.60*	0	5.93*	4.23*	1st
Farm 3 (LH × LB)	−4.33*	−5.93*	0	−1.70*	4th
Farm 4 (LH × HB)	−2.63*	−4.23*	1.70*	0	3rd

**Shows the mean difference is significant at the 0.05 level.*
Adjustment for multiple comparisons: Least Significant Difference.

semantically and thematically. **Figure 2** illustrates the outcome of the semantic analysis, detailing the percentage of positive, negative, and neutral responses within each vignette scenario and the themes that emerged within each sentiment category.

Farm One: Health Issues Minimized × Natural Behaviors Not Promoted

Sentiment analysis indicated a mostly positive response to farm one, with responses including 60% positive sentiments, 37% negative, and 3% neutral. Health being supported was the predominant reason given within positive responses. Here, participants mainly focused on the animals in the scenario being free from pain and stress which, therefore, indicated to them that the animals' main needs would be met "*If the animal is stress, pain and illness free then overall the vast majority of its needs is being met*" (Beef & Sheep, ROI). Several participants also discussed this scenario in terms of the farmer responsible for the animals, perceiving them as an individual who cares for the welfare of their animals; "*The farmer clearly has a concern for the welfare of their livestock*" (Sheep, UK). In sum, positive responses here came from the fact that health issues were minimized, which participants perceived indicated that both the animals' needs were being met and that the farmer appropriately cared for their animals and their welfare.

The negative sentiments (37%) directed toward this scenario predominantly focused on the lack of support for natural behaviors; "*Some indication of appreciation of natural behaviours would have increased my score*" [Pig & Poultry (free-range turkey), UK]. Interestingly, the reasons why natural behaviors were considered important varied between individuals. For some, supporting natural behaviors was considered to support health; "*There are health benefits to ensuring an animal can express natural behaviours*" (Sheep, UK), for others, there were productivity benefits; "*Efforts must be made to allow natural behaviour as it can enhance farm KPIs*" (Pig, ROI). Participants also linked natural behaviors with the mental health of the animal, as described in the following narrative (notable for the level of detail it provides); "*Psychological well-being can have a detrimental or beneficial effect on an animal's physical health. An animal that is consistently deprived of contact with others of its kind, or conversely placed in an overcrowded situation, can stop eating, engage in repetitive adverse behaviour (e.g., tail biting in pigs), adopt pica-like dietary habits, be subjected to bullying and fall foul of perceived idiopathic health issues (infertility, agalactia, wasting syndromes etc.). It is remiss in this age of information to*

TABLE 4 | Impact of significant participant characteristics on judgement variables.

	B	95% CI for *B*		SE *B*	*F*	*df*	*p*	Partial η^2
		LL	UL					
WELL-BEING								
Business type								
Family-run	3.00	0.63	5.37	1.20	6.31	1	0.014	0.061
Commercial partnership	3.95	1.09	6.82	1.44	7.52	1	0.007	0.072
Farming sector								
Beef	1.36	0.24	2.48	0.56	5.82	1	0.018	0.057
PHYSICAL HEALTH								
Business type								
Family-run	2.91	0.51	5.30	1.21	5.82	1	0.018	0.057
Commercial partnership	3.23	0.35	6.12	1.45	4.94	1	0.029	0.048
Farming sector								
Beef	1.25	0.12	2.38	0.57	4.83	1	0.03	0.047
MENTAL HEALTH								
Business type								
Family-run	2.59	0.03	5.15	1.29	4.04	1	0.047	0.04
Commercial partnership	4.46	1.37	7.55	1.56	8.22	1	0.005	0.078
Farming sector								
Dairy	−1.51	−2.98	−0.05	0.74	4.19	1	0.043	0.041
PRODUCTIVITY								
Belief in animal mind	0.34	0.04	0.64	0.15	4.94	1	0.029	0.048
Business type								
Family-run	2.47	0.11	4.82	1.19	4.33	1	0.04	0.043
Farming sector								
Beef	1.39	0.28	2.49	0.56	6.14	1	0.015	0.06
SOCIAL NORMS								
Belief in animal mind	0.32	0.01	0.63	0.16	4.27	1	0.042	0.042

B, Unstandardised regression coefficient; CI, Confidence Interval; LL, Lower Level; UL, Upper Level.

believe that so long as an animal doesn't have a visible or observed affliction that it is entirely healthy, if its psychological needs haven't been met as diligently as its physical ones" (Sheep, Beef & Laying-hen, UK). In addition, some participants proposed ways to support natural behaviors, highlighting the role of environment enrichments; "*Stimulus such as brushes allow animals to groom, improving wellbeing*" (Dairy, ROI), and the support of social interaction; "*Social grouping is key to some species—more than 'entertainment'*" (Sheep, UK). In addition, a small number of respondents expressed negative sentiments toward the farmer in the scenario, describing them as being reactive rather than proactive and for not doing more to promote animal well-being; "*Would not go out of his way to make the animals happy*" (Beef & Dairy, ROI).

A small number (3%) of responses demonstrated neutral sentiments. These were predominantly individuals who, rather than discussing the scenario, discussed their own personal farm, describing their own management practices and what they do to support the health and natural behaviors of their own animals. These included factors such as reducing stress, handling animals calmly, ensuring animals have enough space and comfortable housing, keeping animals in familial groups and numerous health provisions to ensure high health. A small number of responses also expressed a belief that most farmers are doing their best and cannot get it right all of the time; "*Nobody's perfect*" (Sheep, UK), indicating they may have been more forgiving in their ratings of well-being than other participants.

Farm Two: Health Issues Minimized x Natural Behaviors Promoted

Sentiment analysis indicated a large (80%) positive response to this scenario. Discussion here primarily focused on the farmer, describing them as a "good farmer" who prioritized the well-being of their animals: "*These sound like the views of a good farmer, wanting the best for his or her animals*" (Sheep & Laying-hen, UK); "*Animal wellbeing is front and centre of this farmers mind-set*" (Dairy, ROI). The farmer, in the scenario, was perceived to be a "good farmer" because they considered the animal's perspective, understood their needs and/or prioritized the animals in their management decisions: "*Good set up with animals at heart of it*" (Sheep, UK); "*The farmer obviously understands the needs of the animals he/she is caring for*" (Sheep, UK). Several respondents also positively commented on how both health issues were

FIGURE 2 | Sentiment analysis of qualitative responses of reasons given for well-being ratings.

minimized and natural behaviors promoted, highlighting how this would minimize negative factors (e.g., stress) and support positive behaviors (e.g., social interaction): "*Animals are living in a stress free environment and able to choose their preferred behaviours and social contacts*" (Dairy & Sheep, ROI). Several participants expanded on and described how they perceived the scenario was positively supporting natural behaviors, mentioning factors such as animal's having freedom and the opportunity to exert choice; "*The freedom to display natural behaviours and make decisions for themselves*" (Beef, ROI), adequate space provision; "*He gives them space to roam*" (Beef & Sheep, UK), and the importance of social interaction; "*Cattle have strong family and friendships and bonds—they like to see one another and groom/play*" (Beef, Sheep & Dairy, UK). However, some responses mentioned only health issues being minimized when discussing what positively influenced their rating of well-being: "*Emphasis on animal health and low stress*" (Sheep, UK). A small number of responses also associated this scenario with positive affect, describing it in terms of animals being happy; "*Happy Cows*" (Dairy, UK) and contented; "*Animals should be….kept in conditions that allow them to be contented*" (Sheep & Laying-hen, UK). Interestingly, a very small number of participants directly drew on the five freedoms, judging the scenario positively because of a perception that it addressed all five of the freedoms; "*Addresses five freedoms including ability to express natural behaviour*" (Seep & Dairy, UK).

There were a small number of negative sentiments (16%) expressed about this vignette. These were criticisms of the scenario, describing how the factors presented in it do not necessarily lead to better welfare; "*Just because they can wander around and choose where they want to lie doesn't guarantee better well-being*" (Pig, UK). In addition, one respondent cautioned that providing animals with too much choice (e.g., food choice) may have negative effects; "*It seems an excellent scenario but it isn't necessarily sensible to allow animals to eat too much*" (Sheep, UK).

Neutral sentiments (4%) were, again, respondents who discussed their own personal farms rather than the specifics of the scenario. In this case, they mentioned factors such as having an extensive management system (e.g., sheep free to graze on hill land), farming in a traditional way (e.g., non-intensively, with traditional breeds), limiting animal stress (e.g., not using dogs to herd), and a focus on preventative healthcare (e.g., preventing lameness).

Farm Three: Health Issues Not Minimized x Natural Behaviors Not Promoted

Responses to the scenario were overwhelmingly negative (91%) in their sentiment. For the majority, this was due to the lack of intervention in relation to dealing with health issues (i.e., the sore foot). These, many participants argued, should in the very least be investigated, stressing that a lack of intervention could lead to more serious problems arising; "*Many diseases will self-cure but leaving issues such as lameness untreated will eventually lead to larger outbreaks and a greater overall welfare problem*" (Beef & Sheep, UK), and would result in animals being in pain, discomfort or distress; "*You need to intervene to avoid animals being in discomfort*" (Sheep, ROI); "*Animals in pain should be treated to prevent suffering*" (Beef, ROI). Within this, several participants argued that intervention to deal with health issues is particularly important when animals are kept in a non-natural environment; "*I don't like to see my animals suffer unnecessarily, especially if they are living in an unnatural environment, for example on slatted floors*" (Pig, UK), and that humans therefore have a duty to care for their health; "*Animals kept on a farm are removed from nature and must have their health proactively managed by the herd-owner*" (Dairy, ROI).

Many respondents were also critical of the lack of support for natural behaviors in this scenario. Here, participants expressed views that animals should be able to express their natural behaviors; "*Animals should be allowed to exhibit/ experience natural behaviour as much as possible within a normal production system*" (Beef & Sheep, UK), and described different ways in which natural behaviors could be promoted and supported; "*Allowing an animal to express normal behaviour is sometimes about doing nothing. Sometimes it's about doing something, or providing something, i.e., a cow brush, or providing a situation i.e., allowing a hen to scratch for food or a pig to dig*" (Beef & Sheep, UK). Closely related to this, was a view that the extent to which natural behaviors need to be promoted can depend upon the type of system or environment an animal was in: "*For the behaviour it depends what the housing is like—the animals may be in a very extensive system, which doesn't need many specific things to aid natural behavioural expression*" (Pig, UK). One individual also highlighted how a lack of support for natural behaviors may further exacerbate a health issue, describing how; "*If there was no effort to support natural behaviour expression, a sick animal may not have the space and quiet that it might choose to seek out*" (Sheep, UK). Interestingly, one of the criticisms directed toward this scenario was described within terms familiar to the positive welfare literature, where the participant specifically argued that the primary issue with this scenario was a lack of interest in, or support for, positive aspects of welfare: "*'Freedom from pain/suffering' are key parts of the basic requirements for 'A Life Worth Living'. By letting nature take its course this farmer is not abiding by that. Well-being means moving toward 'A Good Life' in all aspects of livestock care. In an unnatural farmed situation we need to ensure animals have the maximum opportunity to express natural behaviour. This farmer is not doing that*" (Sheep & Other (Goat), UK).

The small number of positive responses (5%) to this scenario were primarily based on the fact that only an "odd" animal was described as having a sore foot in the vignette, and was therefore not a whole group problem; "*Only a small proportion of group*" (Sheep, UK). What was particularly notable was how participants considered and weighed up the context they perceived the animals were in, influencing their judgements of the scenario; "*It depends on how long the animal has been lame for. If it has been lame for ten days very maximum, I would get it in to see whether it has an abscess/infection. The reason I gave 5, is that farmer stated the odd animal rather than a number of animals*" (Sheep, UK). A small number of participants also reasoned that a lack of intervention, if the animal was not suffering too much, may be positive if this reduced antibiotic usage; "*I think some farmers are too quick to intervene with antibiotics when we are trying to reduce the usage, so it is good in that respect. But if the animal is in pain, or antibiotics would aid the recovery, then the farmer shouldn't hesitate in jabbing the pig*" (Pig, UK). In addition, some participants felt that an experienced farmer would be able to effectively judge when there is a need to intervene on a health issue; "*An experienced farmer will know when to intervene and when to leave to heal itself*" (Beef & Sheep, UK). Overall, it was evident that positive responses were based on the consideration of specific conditions or contexts which may ameliorate the potential negative impact a lack of intervention may have on animal health.

Neutral responses (4%) were, again, participants who discussed their own personal farm rather than the scenario.

Farm Four: Health Issues Not Minimized × Natural Behaviors Promoted

Responses to this scenario were largely negative (81%). The primary reason for these negative responses was the lack of intervention in dealing with health issues (e.g., the 'sore foot); there was an almost unanimous view that "*The sore foot needs to be looked after*" (Beef, ROI). For many, a lack of intervention was considered poor management and a cause for concern due to the pain and discomfort that may result; "*If an animal is identified with an obvious health problem it should be treated accordingly to ensure maximum comfort is given to the animal to improve its wellbeing*" (Beef, UK). Some even described the situation as cruel, causing suffering or a welfare issue; "*Allowing [animals] to heal naturally with no vet treatment plan is negligent and causes suffering*" (Sheep, UK). Several participants demonstrated strong, personal views on the lack of health intervention, reflecting on how they would approach and deal with the scenario; "*If there is an animal lame, I wouldn't leave it untreated, I would have to check and see what the problem was, it could be something as* *simple as a stone in the hoof and by removing it you're taking away that chance of it developing into a more painful situation for the animal*" (Beef & Dairy, ROI). In addition, respondents also expressed concerns that a lack of intervention could lead to the condition worsening or more serious health problems; "*You need to intervene with animal health and welfare i.e., sore feet. Leaving it alone can cause more health issues*" (Sheep. UK). Such negative views of the lack of health intervention further contributed to the view that, at the very least, the health issue should be investigated before any decisions made (e.g., to leave to heal naturally or intervene); "*The farmer could have at least examined the foot of the animal rather than observe from a distance. It could have just needed debris removed or a natural treatment could have been administered*" (Sheep & Poultry Other, UK). A small number of participants also expressed the view that, because farm animals are domesticated and rely on human intervention, they are responsible for their well-being and therefore are duty-bound to address health issues; "*Livestock are domestic animals and early intervention is often necessary*" (Sector unknown, ROI); "*I believe when animals are in your care, you should treat any discomfort they have no matter how small*" (Sheep, UK). Interestingly, one participant constructed their criticism of a lack of intervention on health issues through its effect on productivity; "*I think it unlikely that a commercial farmer would leave an animal with a 'sore foot' to 'heal itself' as such animals are less likely to be able to feed well and grow well enough to be profitable*" (Sheep, UK). Thus, overall, respondents viewed this scenario negatively because health issues were not minimized, expressing concerns for the negative impact this would have on animal well-being, its potential to cause suffering or lead to greater health issues and considering it a sign of poor management or neglect.

Despite the large portion of negative responses, participants also demonstrated positive responses (9%), predominantly due to the support for natural behaviors which participants commended and considered a positive contributor to animal well-being; "*Allowing animals to express normal behaviour should always be encouraged and this contributes to well-being in a positive way*" (Sheep, UK). Within this, respondents largely focused on opportunities for animals to be outside; "*I like animals to have access to outside when suitable*" (Beef, UK), describing this as a natural environment for animals; "*Good appreciation of animals need for habitat and natural behaviour*" (Beef & Sheep, UK), and emphasized the benefits of animals having space and opportunities for social interaction "*Plenty of space and exercise is beneficial for the animals' mental and physical health, they are able to get away from animals that pick on them and are able to socialise freely*" (Beef, UK). In addition, a very small number of respondents positively focused on "letting nature take its course" in regards to dealing with health issues. Specifically, they felt that some intervention would be necessary but agreed with the general attitude; "*Sore foot unlikely to heal on its own, however letting nature take its course is almost always best*" (Beef & Sheep, UK).

Despite these positive sentiments toward this scenario, due to natural behaviors being supported, perceptions of it were largely negative. Within their discussions, participants gave an indication as to why it was viewed so negatively, despite natural behaviors being supported. Namely, they described and viewed health as the priority; "*The second part of the sentence I'd agree with, it suggests good well-being, but health is a primary importance and it seems the farmer ignores small indicators of ill-health*" (Sheep & Poultry Other, UK); "*Animal health should be a priority*" (Beef, UK). Such responses suggest that participants give precedence to health such that, in a situation where there is a trade-off between health and natural behaviors, animal well-being cannot be considered positive if only natural behaviors are supported.

There were also neutral responses to this scenario (10%). Again, these were responses where participants discussed their own personal farm, describing factors such as how out-wintering their animals results in lower levels of disease and health-issues or how they treat health issues on their own farm. In addition, several also discussed how the impact of a health issue on animal well-being may depend on the context or situation the animal is in. For instance, the number of animals the farmer may have to manage; "*Farmer sounds inclined to neglect however the size of the operation and type of animal may make the individual care of one foot impractical*" [Laying-hen (free range), UK], or how intervening may cause stress for a greater number of animals; "*Understandably catching a whole flock to treat one lame sheep can cause stress, but that one lame sheep can then spread the issue to other animals in the flock without intervention*" (Beef & Sheep, UK). Thus, a weighing up of different contextual factors which may influence their decision was evident in participants' discussions and judgements of this scenario.

Qualitative Responses: What Would Participants Change About the Scenario?

To gain an insight into what participants considered may be lacking or needed improving within each scenario, they were asked to describe what they would change about the scenario they were assigned to. Findings are presented in **Figure 3**, which illustrates the content analysis of responses in a wordcloud, whereby the most common responses are represented by the largest word. Responses reflect and support the views expressed in the previous section. Specifically, that there is a need for more support of natural behaviors in farm one (HH × LB), that farm two (HH × HB) was largely an excellent scenario where "nothing" needed to be changed and that both farm three (LH × LB) and farm four (LH × HB) required a consideration of health and intervention to minimize health issues.

Overall Attitude to the Importance of Minimizing Health Issues and Promoting Natural Behaviors

To assess participants' overall attitude to the importance of minimizing health issues and promoting natural behaviors for animal well-being, they were asked to select which of these factors they thought was most important and, in addition, rate (on a scale from 0 to 10) the importance of each. The majority of participants (87.6%) considered "minimizing health issues" to be the most important factor for animal well-being. When asked to rate how important each factor was, "minimizing health issues"

FIGURE 3 | Wordcloud of qualitative responses to what participants would change about vignette scenarios.

was rated slightly higher ($M = 9.69$, $SD = 0.60$) than "promoting natural behaviors" ($M = 8.57$, $SD = 1.53$). A paired sample t-test indicated that the difference in ratings was statistically significantly different, with minimizing health issues considered more important for overall well-being, than promoting natural behaviors by 1.124 (95% CI, 0.899–1.349); $t_{(169)} = 9.870$, $p < 0.001$; Cohen's $d = 0.76$.

Factors Impacting Ratings of the Importance of Health and Natural Behaviors

For the importance of minimizing health issues, none of the predictor variables had a significant effect on participants' ratings of this variable.

For the importance of promoting natural behaviors, gender (Wald $x^2_{(1)} = 4.18$, $p = 0.041$) and the way in which participants managed their animals (i.e., management system) (Wald $x^2_{(5)} = 11.22$, $p = 0.047$) were found to be significant predictors. However, these were not adjusted for multiple testing (due to constraints within the functionality of SPSS) and given their closeness to the $p < 0.05$ significance threshold, it is likely they would not remain significant if adjusted for other predictors. Nevertheless, the direction of their effect is interesting. Males were significantly less likely to give a higher rating for the importance of promoting natural behaviors than females, with an odds ratio of 0.38 (95% CI, 0.15–0.96). In other words, males were more likely to give a lower rating for the importance of natural behaviors than females. Participants who kept the majority of their animals outdoors all year round were 3.97 times (95% CI, 1.24–12.75) more likely to give higher ratings for the importance of promoting natural behaviors than participants who housed animals for part-of the year and kept them outdoors part-of the year; a significant effect, Wald $x^2_{(1)} = 5.38$, $p = 0.020$. The information participants received on health and behavior provision in the vignette scenarios were not significant predictors of attitudes to the importance of natural behaviors.

DISCUSSION

This study set out to examine the importance farmers give to minimizing health issues and promoting natural behaviors within their welfare-related attitudes and judgements, and the extent to which individual differences may influence this. The findings of this study are broadly in line with and support previous research which finds that farmers prioritize the minimization of health issues within their conceptions of animal welfare (e.g., Bourlakis et al., 2007; Kauppinen et al., 2010). When asked to make a choice between minimizing health issues and promoting natural behaviors, the majority of participating farmers selected minimizing health issues as the most important factor for animal well-being. Furthermore, when asked to rate how important they considered each to be for animal well-being, participants' attitudinal responses indicated that minimizing health issues was considered slightly more important for animal well-being than promoting natural behaviors. Importantly, beyond these attitudinal factors, this study also found that the information provided on health provision (i.e., whether health issues were minimized or not) in the vignette scenarios had more of an impact on participants' judgements than any other variable, including participant characteristics. Thus, how an animal's health is being managed appears to be a central consideration within farmers' welfare related decision-making. Previous research has demonstrated a connection between farmers' attitudes and their welfare-related behavior and judgements, with the former often considered to predict the latter (Hansson and Johan Lagerkvist, 2012). As such, the impact of health provision on participants' judgements of the vignette scenarios could be explained by a pre-existing attitude that health is the most important factor for animal well-being. Within the context of a changing policy landscape, this is a particularly important finding for understanding how farmers' perspectives may contribute to them having different welfare priorities from other key stakeholders (e.g., public). However, as the vignettes provided information only on health and natural behaviors, it would be erroneous to suggest that health provision is the primary consideration for farmers in every welfare-related situation. Rather, as Kristensen and Jakobsen (2011) suggest, farmers' judgement and decision-making is likely to be context-bound where, in the context of the presented vignettes, health was judged to be the factor of primary importance.

It is notable that welfare was judged most positively when both health issues were minimized and natural behaviors promoted, as revealed by the farm two (HH × HB) scenario receiving the most positive (i.e., most highly rated) judgements. This suggests that, while health may play a central role in their judgements of welfare, what farmers want is for animals to be both healthy and able to express their natural behaviors. Notably, participants' qualitative responses to scenarios where natural behaviors were not promoted (i.e., farm one and farm three), criticized the lack of support for natural behaviors. They also suggested ways in which natural behaviors could be supported (e.g., providing enrichment, social interaction), further revealing how they view and construct what is relevant for natural behaviors. Moreover, when asked what they would change about such scenarios, greater promotion of natural behaviors was the most common response to those assigned to low behavior (i.e., natural behaviors not promoted) vignettes. As such, findings suggest that farmers also care about natural behaviors, or at least recognize a lack of support for them as an issue, and are knowledgeable of mechanisms and means to enable them. Natural behavior provision also had a significant impact on participants' judgements of the different welfare attributes in the vignette scenarios (albeit less than health provision but more than any personal characteristics). Interestingly, compared to its impact on other judgement variables, natural behavior provision most strongly impacted judgements of animal mental health. This suggests that different welfare-relevant provisions (e.g., health, natural behaviors) may be perceived by farmers to have different roles or be important for different aspects of an animal's welfare. That farmers appear to consider natural behaviors particularly relevant to animal mental health is in line with much of the positive welfare literature, which argues that for animals to experience positive affect they require opportunities to engage in positive experiences (e.g., Boissy et al., 2007; Mellor, 2012). As such, the findings of this study suggest that both health and natural behaviors matter to farmers. Such a finding adds to the debate on the potential animal welfare consequences of increasing the efficiency of animal production to address sustainability and climate change (e.g., Shields and Orme-Evans, 2015; Clay et al., 2020); it may also be at odds with farmers' personal welfare expectations and values relating to natural behavior expression.

In considering our findings together, it is possible to suggest that farmers view and judge the importance of these two factors of welfare on a continuum. Participants' judgements, when there was a trade-off between the provision of health and natural behaviors, provide further support for this. Welfare-related judgements were always rated lower when health issues were not minimized, even if natural behaviors were simultaneously supported in the vignette scenarios. It is thus possible to theorize that farmers, when simultaneously presented with information on health and natural behaviors, may first look to health provision as a criteria for good welfare. In situations where health is not taken care of but natural behaviors are, the former may be perceived to offset any good done by the latter. Indeed, several participants emphasized in their qualitative responses that natural behaviors are important but that dealing with health issues should be a priority. Such views somewhat echo perspectives which place animal experience on a continuum from "pains" to "pleasures" (Fraser and Duncan, 1998), with farmers situating their primary role as that of minimizing "pains" so that the animal is free and able to engage in "pleasures" of their own accord (Vigors and Lawrence, 2019). Indeed, the views and judgements of participating farmers are not that different to those found by Duncan (1996, cited in Fraser and Duncan, 1998) who concluded that minimizing suffering is the main priority, where enabling animals to engage in normal behaviors and "pleasures" is also considered important but of lower priority than the former. In short, our findings indicate farmers consider both health and natural behaviors to be important for welfare, judging welfare to be "best" when both are supported, but, when there is a trade-off between the two, minimizing health issues takes priority.

When individual characteristics are accounted for, interesting nuances in attitudes toward the importance of health and natural behaviors and their impact on judgements of welfare are notable. This supports previous findings which suggest greater heterogeneity amongst farmers in attitudes to natural behaviors (e.g., Bourlakis et al., 2007; Skarstad et al., 2007; Spooner et al., 2014b). For instance, gender was found to influence attitudes to health and natural behaviors, with female participants found to give higher ratings for the importance of natural behaviors than males. This is line with a large body of research on the effects of gender on attitudes to animal welfare, where females are largely known to be more concerned by animal welfare (Herzog, 2007; Apostol et al., 2013; Clark et al., 2016). However, much of this research focuses on members of the public, with few studies examining the impact of gender on farmers' welfare-related attitudes. This study is notable for its large proportion of female participants (47%), perhaps reflective of a noted increase in female participation in the agriculture workforce in recent years [from 26.3% in 2005 to 28.4% in 2016 in the EU, see DaSilva et al. (2018)]. However, the gender configuration of this study is not representative of the agricultural workforce, where (in 2016) 15% of farmers in the UK and 10.8% in the Republic of

Ireland were female (DaSilva et al., 2018). The high proportion of females choosing to participate in this study may thus be a further example of the greater interest females take in animal welfare.

Interestingly, participants who kept their animals outdoors all year round were more likely to give higher ratings for the importance of promoting natural behaviors. This suggests that participants with such a system, where animals arguably have greater opportunity to engage in natural behaviors, place greater emphasis on the importance of natural behaviors within their conceptions of welfare. Such findings support suggestions in the literature that farmers' attitudes to welfare may be influenced by their production system or management practices (Bourlakis et al., 2007; FAWC, 2011; Spooner et al., 2014b) and that differences in production systems are associated with differences in attitudes to welfare (Kjaernes et al., 2007). However, the converse may also be possible, where farmers' may choose management and production systems that are in line with and reflect their values and definitions of animal welfare (Dockès and Kling-Eveillard, 2006; Vigors and Lawrence, 2019). Whatever the direction of this effect, the findings do suggest that the type of production or management system a farmer has, influences the emphasis they place on health and natural behaviors. However, analysis of the effects of gender and management system on attitudes to natural behaviors were not adjusted for multiple testing. Consequently, they may not remain significant at the 5% level if adjusted for other predictors. The direction of their effect is thus important but should be interpreted with caution.

Type of farm-business also impacted judgements of the vignettes, where having a family-run business was associated with more positive judgements of well-being, physical and mental health, and productivity, and having a commercial partnership was associated with more positive judgements of well-being, physical health, and mental health. Few studies have directly investigated the impact of business type on welfare-related decisions making it difficult to interpret the potential reasons for this finding. However, Macken-Walsh et al. (2012), in an in-depth qualitative study into the experiences of Irish beef farmers on family-run farms, explicitly describes the "sense of well-being and satisfaction [they] attained from their interactions with and care of livestock" (p. 10). The care they provided to their animals was a key source of personal enjoyment, intertwined with their self-identity and self-worth. In addition, family-run farms are characterized by farmers and their family as the primary animal care-givers and primary source of labor (Gray, 1998) which may mean they are more likely to know individual animals and have direct and regular interaction with their livestock. As such, the cultural capital family-run farms ascribe to caring for livestock (Macken-Walsh et al., 2012) and the attitudes they may develop from directly working with their animals may have influenced how participants from family-run farms judged welfare in the vignette scenarios. Although there has been research into the economic and social benefits of commercial farm partnerships (e.g., Macken-Walsh and Roche, 2012), this has not examined the impact of such partnerships on the farmers' relationship with or attitudes to their animals' welfare. Consequently, it is difficult to suggest what may underlie the more positive judgements of well-being, physical and mental health given by participants with a commercial partnership, beyond noting its effect.

The type of farming sector also had an impact on participants' judgements. Specifically, participants from the beef sector judged well-being, physical health and productivity more positively. Beef farming in the UK and Ireland is largely characterized by extensive grassland systems (Rath and Peel, 2005; Hennessy et al., 2018). As such, participating beef farmers potentially judged the vignette scenarios from the personal viewpoint of animals with outdoor access, opportunities to form social interactions, exert some agency and express natural behaviors and therefore, may perceive that direct intervention to promote natural behaviors is not necessary (Vigors and Lawrence, 2019). Thus, they may be more likely to judge the well-being, physical health, and productivity of the animals more positively than farmers from other sectors. Nevertheless, sheep producers in the UK and Ireland also have a similar extensive production system, yet a similar effect was not noted for their judgements. However, it is important to note that "beef sector" was one of the largest samples in this study. As such, comparisons between it and other sectors should be interpreted with caution.

Interestingly, participants in the dairy sector judged mental health to be lower than those in other farming sectors. Previous research on UK dairy farmers' attitudes to animals found that 90% thought cows had feelings and 78% considered cows to be intelligent (Bertenshaw and Rowlinson, 2009). Notably, Hansson and Lagerkvist (2016) found that, when it came to animal-welfare related decision-making, dairy farmers were more motivated by non-use values (i.e., those relating to animal well-being) than by use-values (i.e., those relating to economic output, such as productivity). Indeed they argued that their findings "suggest that profitability of the business is important but that the absolute rights the animals are assumed to have and the feelings of happiness associated with treating animals well are equally, or even more important" (Hansson and Lagerkvist, 2016, p. 590). Thus, it is possible that dairy farmers may give greater consideration to animal mental health—due to its potential relevance to non-use welfare attributes, the value they place on these and their perception of cows as sentient—resulting in them judging the mental health of the animals in this study more critically than farmers from other sectors. However, without further research it is difficult to determine the nature and source of this association between dairy farmers and judgements of animal mental health.

Finding differences between farming sectors in how they judged or appraised aspects of welfare is important; it highlights how different sub-sets of farmers may have different priorities for welfare influenced by the norms and characteristics of their sector and system. This, arguably, may impact how different sectors respond to policies put forth to address wider societal issues. For example, a recent proposal by the UK National Beef Association to introduce a "carbon tax" for cattle slaughtered later than 27 months of age received criticism from many beef farmers, arguing it would discourage less intensive and regenerative farming practices (e.g., mob grazing) (Riley and Price, 2020). As discussed, the norm for beef farmers in the UK is extensive grassland systems and they perceive that opportunities for animals to express natural behaviors are inherent in such a system (Vigors and Lawrence, 2019). When such sector norms and characteristics are considered it is thus possible to understand the negative response of some farmers' to the "carbon-tax" proposal. By revealing potential differences between sectors in how farmers judged different aspects of welfare (and therefore the emphasis they may place on them), the findings of this study are particularly relevant to policy discussions which seek to relate animal welfare to sustainability and climate change. Interestingly, neither membership of assurance scheme nor country (i.e., UK, ROI) were found to explain any of the variance in participants' vignette judgements or their attitudes to health and natural behaviors. However, a greater proportion of Irish participants were members of assurance schemes. This perhaps reflects differences in assurance scheme structure between the two countries, where the Irish context is largely characterized by a singular, state-run assurance scheme while the UK is characterized by numerous, market-based assurance schemes. As such, differences in policy (i.e., between country) do appear to impact participant characteristics but were not found to effect participants' judgements and attitudes. Nevertheless, future research examining differences in health and natural behavior perspectives between farmers operating under different policy conditions would be helpful, potentially enabling a better understanding of how policy could impact farmers' judgement and decision-making.

BAM was also found to have an impact on judgements of productivity, with participants with a higher BAM judging productivity more positively than those with a lower level of BAM. This is somewhat surprising, as it appears to suggest that participants who believe more strongly in animals as sentient beings believe they are also more capable of being productive under varying conditions of health and natural behavior provision. However, in a study of human–livestock relations in Scotland, Wilkie (2005) reports how commercial livestock production is characterized by livestock viewed as "sentient commodities." That is, farm animals are paradoxically positioned by their caretakers as both units of production and "co-workers" capable of feeling, thought and a life of their own (Wilkie, 2005). As such, it is possible that productivity and belief in animal mind may operate in parallel within farmers' perceptions of their animals, were those who believe more in an animal's mental capacity also give greater credence to their ability to be productive under varying conditions. In addition, there is research to suggest that positive human–animal interactions increase productivity, where positive attitudes to animals (e.g., viewing them as intelligent, capable of feeling) are a reliable predictor of such positive behaviors (Bertenshaw and Rowlinson, 2009). Thus, it may also be possible that the participants, in this study, with a greater belief in animal mind engage in more positive human–animal interactions and experience higher levels of productivity on their own farm. Consequently, they may have judged the vignette scenarios in this study through the lens of their own personal experiences and more positively rated the ability of the animals to be productive. Without additional research, however, it is difficult to satisfactorily account for this finding.

This study demonstrates that farmers do place considerable importance on both minimizing health issues and promoting natural behaviors within animal welfare, where the former is largely given priority over the latter but variances in this exist, often due to individual differences. As such, this study largely supports the view that farmers perceptions of animal welfare are diverse (Kirchner et al., 2014) and vary according to differences in production systems (Bock and van Huik, 2007; Spooner et al., 2012) and attitudes to animals (Hanna et al., 2009). However, the findings of this study are limited by their lack of generalisability to the UK and Irish livestock farmer population and by how health and behavior provision were described in the vignette judgement tasks. The sample size was small and dominated by beef and sheep farmers with extensive systems. Consequently, relating the findings of this study to intensive farming systems and related sectors (e.g., pig and poultry) should be done with caution, particularly as the large representation of extensive systems in the sample may have impacted responses relating to natural behaviors. Moreover, as discussed above, the large proportion of female respondents is not representative of gender in livestock farming, potentially influencing responses as females are known to be more sympathetic toward animal welfare (Clark et al., 2016). The vignettes were created using phrases, sentences, and terms used by livestock farmers when describing health and natural behaviors, collected in a previous qualitative study (see Vigors and Lawrence, 2019).This was done to ensure the vignettes were reflective of real-world situations whilst also being phrased in the language farmers use. However, this also means that the aspects of health and natural behaviors presented to participants do not fully reflect how health and natural behaviors are constructed within the welfare science literature. In addition, it is difficult to account for how individual participants may have interpreted "health" and "natural behaviors." Although a descriptor of what they can be taken to mean was included alongside the vignette narratives, it is possible that, given the diverse nature of the sample (e.g., multiple sectors), participants interpreted these terms differently potentially impacting their responses. Nevertheless, the societal importance of natural behaviors remains; while a public and consumer demand for natural behaviors continues to exist, understanding farmers' point of view, and therefore their ability to respond to public expectations arguably remains important, particularly in the context of wider policy debates on "sustainable intensification," food security, and climate change (e.g., Garnett et al., 2013; Shields and Orme-Evans, 2015).

CONCLUSION

The findings of this study provide a clearer understanding of the importance farmers give to health and natural behaviors and how different levels of their provision impact farmers' judgements of welfare. In addition, it contributes to research which finds greater heterogeneity in farmers' attitudes to natural behaviors by providing insights on individual characteristics which may account for these differences. Overall, findings indicate that farmers value both health and natural behaviors and judge welfare to be at its best when both are supported. However, findings do suggest that priority is given to minimizing health issues and this appears to be central to farmers' welfare-related judgements. Such findings are of particular significance in light of growing scientific and societal interest in supporting positive aspects of welfare and the impact of this for farmers' social license to farm. Although health is a priority issue for farmers, they may increasingly need to demonstrate the importance they give to natural behaviors. Critically, this study also highlights the relevance of individual characteristics when seeking to understand how farmers approach and judge aspects of welfare, particularly natural behaviors. Arguably, a farmer's attitudes toward welfare and their welfare-related decision-making cannot be separated from their personal values, beliefs and experiences. Such insights may become increasingly important for policy debates on climate change and sustainability—farmers' positive or negative response to policies aimed at addressing sustainability and climate change (e.g., increasing efficiency) may be impacted by their welfare priorities—highlighting the growing need for animal welfare to inform and be integrated into these debates.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Social Science Ethics Committee, Scotland's Rural College, Edinburgh, UK. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BV: conceptualization, methodology, formal analysis, investigation, writing–original draft, and visualization. DE: validation and formal analysis. AL: conceptualization, methodology, writing-review and editing, funding acquisition. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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frontiers in Animal Science

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Individual Detection and Tracking of Group Housed Pigs in Their Home Pen Using Computer Vision

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Modern welfare definitions not only require that the Five Freedoms are met, but animals should also be able to adapt to changes (i. e., resilience) and reach a state that the animals experience as positive. Measuring resilience is challenging since relatively subtle changes in animal behavior need to be observed 24/7. Changes in individual activity showed potential in previous studies to reflect resilience. A computer vision (CV) based tracking algorithm for pigs could potentially measure individual activity, which will be more objective and less time consuming than human observations. The aim of this study was to investigate the potential of state-of-the-art CV algorithms for pig detection and tracking for individual activity monitoring in pigs. This study used a tracking-by-detection method, where pigs were first detected using You Only Look Once v3 (YOLOv3) and in the next step detections were connected using the Simple Online Real-time Tracking (SORT) algorithm. Two videos, of 7 h each, recorded in barren and enriched environments were used to test the tracking. Three detection models were proposed using different annotation datasets: a young model where annotated pigs were younger than in the test video, an older model where annotated pigs were older than the test video, and a combined model where annotations from younger and older pigs were combined. The combined detection model performed best with a mean average precision (mAP) of over 99.9% in the enriched environment and 99.7% in the barren environment. Intersection over Union (IOU) exceeded 85% in both environments, indicating a good accuracy of the detection algorithm. The tracking algorithm performed better in the enriched environment compared to the barren environment. When false positive tracks where removed (i.e., tracks not associated with a pig), individual pigs were tracked on average for 22.3 min in the barren environment and 57.8 min in the enriched environment. Thus, based on proposed tracking-by-detection algorithm, pigs can be tracked automatically in different environments, but manual corrections may be needed to keep track of the individual throughout the video and estimate activity. The individual activity measured with proposed algorithm could be used as an estimate to measure resilience.

Keywords: tracking, computer vision, pigs, video, activity, resilience, behavior

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INTRODUCTION

Successful adaptation to changes is beside the Five Freedoms a critical pillar in modern animal welfare definitions (Mellor, 2016). Animals should be able to cope with challenges in their environment and reach a state that the animals experience as positive. In other words, to enhance pig welfare, pigs should be not only free from any kind of discomfort but also be resilient to perturbations. Resilient pigs are able to cope or rapidly recover from a perturbation (Colditz and Hine, 2016). Perturbations in pig production could be management related (e.g., mixing, transport) or environment related (e.g., disease, climate). Non-resilient pigs have more difficulty recovering or cannot recover at all from perturbations and therefore experience impaired welfare. The lack of ability to cope with perturbations causes a risk for these non-resilient animals to develop intrinsic problems like tail biting or weight loss (Rauw et al., 2017; Bracke et al., 2018).

To prevent welfare problems in pigs, a management system that provides information on resilience will most likely be needed in the future. With such a management system, the farmer will know when resilience is impaired and which animals it concerns. These animals labeled by a management system as non-resilient could be assisted when required. However, such a system is difficult to develop since resilience is difficult to measure. Resilience consists of many parameters that could be monitored. Currently, mainly physiological parameters are used to measure resilience in pigs. Blood parameters such as white blood cell count or hemoglobin levels, but also production parameters such as body weight are used to measure resilience (Hermesch and Luxford, 2018; Berghof et al., 2019). However, measuring these physiological parameters requires invasive handling of the animal. In addition, these parameters represent a delayed value due to the nature of the measurements, and therefore they are less suitable for immediate decision support.

Recent studies investigated activity and group dynamics as traits to measure resilience. Several studies show a reduction in activity as a response to sickness (van Dixhoorn et al., 2016; Trevisan et al., 2017; Nordgreen et al., 2018; van der Zande et al., 2020). Pigs are lethargic during sickness; they spend more time lying down and less time standing and feeding. Not only sickness affects activity, but also climate has an influence on the activity of pigs, with pigs showing lower activity levels when temperature increases. Costa et al. (2014) showed that relative humidity affected pig activity as well and that pigs had a preference to lay close to the corridor when relative humidity was high. To conclude, activity could be a suitable indicator of resilience to perturbations of different nature.

It is extremely time-consuming to measure activity and location of individuals in multiple pens continuously by human observations. The use of sensors could facilitate automatic activity monitoring and minimize the need for human observers. The activity of an individual could be measured by using accelerometers, which could be placed in the ear of the pig, just like an ear tag. Accelerometers measure accelerations along three axes. With the use of machine learning, accelerations can be transformed into individual activity levels (van der Zande et al., 2020). The main advantage of using accelerometers is that the devices usually have a static ID incorporated in their hardware. In other words, identities of animals are known all the time unless they lose the accelerometer. On the other hand, accelerometer placement could affect readings and therefore introduce extra noise in raw acceleration data. With placement in the pig's ear, ear movements can cause confounding of true levels of physical activity. Noise in acceleration data could lead to false positive activity. In addition, the location of the animal is not known when using accelerometers, which further limits more precise resilience measurements since proximity and location preference could be included when the location is known.

As an alternative to sensors placed on animals, computer vision allows for non-invasive analysis of images or videos containing relevant individual activity and location data. Several studies investigated computer vision algorithms to recognize a pig and track it in a video to estimate activity (Larsen et al., 2021). The main advantage of using computer vision to measure activity is that activity is calculated from the pig's location in each frame, allowing for the calculation of proximity to pen mates and location preferences. Ott et al. (2014) measured activity on a pen level by looking at changes in pixel value between consecutive frames of a video. They compared the automated measured activity with human observations and found a strong correlation of 0.92. This indicated that the use of algorithms for automated activity monitoring could minimize the need for human observations. The limitation of the approach of Ott et al. (2014) is that in their method, the activity is expressed at pen level, where individual information is preferred for a management system. Pigs observed from videos are difficult to distinguish individually, so Kashiha et al. (2013) painted patterns on the back of pigs to recognize individuals. An ellipse was fitted to the body of each pig, and the manually applied recognition pattern was used to identify the pig. On average, 85.4% of the pigs were correctly identified by this algorithm. Inspired by patterns, Yang et al. (2018) painted letters on the back of the pigs and trained a Faster R-CNN to recognize the individual pigs and their corresponding letters. Tested on 100 frames, 95% of the individual pigs was identified correctly. These studies mainly concentrated on detecting the manually applied markings/patterns for pig identification and while the approach showed relatively good performance, the manually applied marking is labor intensive. Markings must be consistent and at least be refreshed every day to be able to see the markings properly.

Huang et al. (2018) used an unspecified pig breed with variation in natural coloration and made use of this natural variation to identify pigs from a video. A Gabor feature extractor extracted the different patterns of each individual and a trained Support Vector Machine located the pigs within the pen. An average recognition of 91.86% was achieved. However, most pigs in pig husbandry do not have natural coloration. Another possibility is to recognize individuals by their unique ear tag (Psota et al., 2020). Pigs and ear tags were detected by a fully-convolutional detector and a forward-backward algorithm assigned ID-numbers, corresponding to the detected ear tags, to the detected pigs. This method resulted in an average precision >95%. Methods using manual markings are successful but could still be invasive to the animal and labor intensive.

The studies that do not rely on manual marking of animals, have difficulties in consistent identification of individuals during the tracking. Ahrendt et al. (2011) detected pigs using support maps and tracked them with a 5D-Gaussian model. This algorithm was able to track three pigs for a maximum of 8 min. However, this method was also computationally demanding. Cowton et al. (2019) used a Faster R-CNN to detect pigs at a 90% precision. To connect the detections between frames (DEEP) Simple Online Realtime Tracking (SORT) was used. The average duration before losing the identity of the pig was 49.5 s and the maximum duration was 4 min. Another method used 3D RGB videos rather than 2D RGB videos to track pigs (Matthews et al., 2017). Pigs were detected with the use of depth data combined with RGB channels, and a Hungarian filter connected the detected pigs between frames. The average duration of a pig being tracked was 21.9 s. Zhang et al. (2018) developed a CNN-based detector and a correlation filter-based tracker. This algorithm was able to identify an average of 66.2 unique trajectories in a sequence of 1,500 frames containing nine pigs. Despite the variety of methods, none could track a pig while maintaining the identity for longer than 1 min on average. In practice, this would result in a human observer correcting IDs more than 360 times for an hour-long video with six pigs being monitored. A computer vision algorithm used to measure activity should be able to maintain identity for a longer period of time to lower human input.

All the previous studies based on different convolutional neural network (CNN) architectures showed a robust performance when it comes to single pig detection. However, continuous detection across several frames and under varying conditions remains challenging. You Only Look Once v3 (YOLOv3) is a CNN with outstanding performance (Benjdira et al., 2019). SORT could be used for tracking across several frames. SORT is an online tracker which only process frames from the past (and not from the future). The main advantage of an online tracking algorithm is improved speed, but this algorithm is fully dependent of the quality of the detections. The fast and accurate detections of YOLOv3 and the connection of the detections across frames by SORT might allow for longer tracking of individual pigs. Therefore, the aim of this study was to investigate the potential of state-of-the-art CV algorithms using YOLOv3 and SORT for pig detection and tracking for individual activity monitoring in pigs.

MATERIALS AND METHODS

Ethical Statement

The protocol of the experiment was approved by the Dutch Central Authority for Scientific Procedures on Animals (AVD1040020186245) and was conducted in accordance with the Dutch law on animal experimentation, which complies with the European Directive 2010/63/EU on the protection of animals used for scientific purposes.

Animals and Housing

A total of 144 crossbred pigs was used in this study. The pigs originated from the same farm but were born and raised in two different environments: a barren and an enriched environment. Piglets from the barren environment were born in farrowing crates, and the sow was constrained until weaning at 4 weeks of age. Upon weaning, eight pigs per litter were selected based on body weight and penned per litter in pens with partly slatted floors until 9 weeks of age. A chain and a jute bag were provided as enrichment. Feed and water were provided ad-libitum. The second environment was an enriched environment, where piglets were born from sows in farrowing crates. After 3 days post-farrowing, the crate was removed, and the sow was able to leave the farrowing pen into a communal area consisting of a lying area, feeding area, and a dunging area together with four other sows. Seven days post-farrowing, the piglets were also allowed to leave the farrowing pen into the communal area and were able to interact with the four other sows and their litters. The piglets were weaned at 9 weeks of age in this system.

All pigs entered the research facility in Wageningen at 9 weeks of age. The pigs originating from the barren environment remained in a barren environment. Each barren pen (0.93 m^2/pig) had a partly slatted floor and a chain and a ball were provided as enrichment. The pigs originating from the enriched environment were housed in enriched pens (1.86 m^2/pig) which had sawdust and straw as bedding material. A jute bag and a rope were alternated every week. Once a week, fresh peat was provided, as were cardboard egg boxes, hay or alfalfa according to an alternating schedule. Additionally, six toys were alternated every 2 days. Each pen, independent of environment, consisted of six pigs, balanced by gender, and feed and water were available *ad-libitum*. Lights were on between 7:00 and 19:00 h and a night light was turned on between 19:00 and 7:00 h. The experiment was terminated at 21 weeks of age.

Data

An RGB camera was mounted above each pen and recorded 24 h per day during the experiment. The videos were 352 by 288 pixels and recorded in 25 fps. Due to the smaller width of the barren pens, neighboring pens were visible on the videos of the barren pens. To avoid that the pigs from neighboring pens were detected and allow an equal comparison between the barren and enriched environment, the neighboring pens were blocked prior to the analysis (**Figure 1A**). Frames were annotated using LabelImg (Tzutalin, 2015). The contours of the pig were labeled by a bounding box, where each side of the bounding box touches the pig (**Figure 1**). One annotation class (pig) was used, and only pigs in the pen of interest were annotated.

Three different detection models were evaluated to assure the best detection results possible under varying circumstances: using frames where young pigs were annotated (young model), using frames where old pigs were annotated (old model), and a combination. The young model contained annotations of randomly selected frames from pigs around 10 weeks of age. The training dataset consisted of 2,000 annotated frames, where 90% of the frames was used for training, and 10% was used for validation. The old model was trained on 2,000 annotated

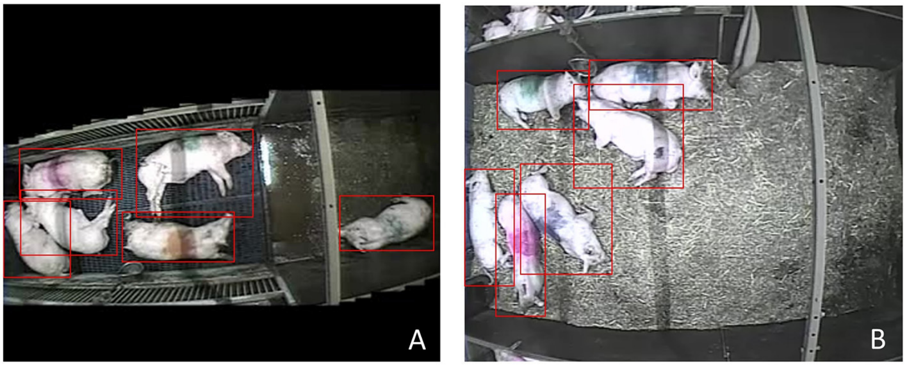

FIGURE 1 | Example frames with annotated bounding boxes (red boxes) in the barren **(A)** environment with blocked neighboring pens and the enriched **(B)** environment.

randomly selected frames of pigs from 17 to 21 weeks of age, where 90% was used for training, and 10% was used for validation. The combined model consisted of young and old animals' annotations, with 4,000 annotated frames split into 90% training data and 10% validation data.

To review a possible difference in the performance of tracking between environments, one video of ∼7 h (n frames = 622,570) of each environment without any human activity except for the activity of the caretaker was used for tracking. The pigs were 16 weeks of age in this video, which is an age that was not used for training of the detection models. Every 1780th frame was annotated to obtain 350 equally distributed frames per environment and to evaluate the three different detection models (young, old and combined). All the frames were then used to test the success of the multiple-object tracking.

Detection Method

To assure high computational speed and robust multiple object detection, the You Only Look Once version 3 (YOLOv3) algorithm was used to detect pigs in their home pens (Redmon and Farhadi, 2018). YOLOv3 is an accurate object detection network that features multi-scale detection, a more robust feature extraction backbone compared to other convolutional neural networks (CNN)-based detectors and an improved loss function calculation. The YOLOv3 framework consists of two main multi-scale modules: the Feature Extractor and the Object Detector (**Figure 2**). The input for YOLOv3 are frames/images of interest. First, an input frame/image passes through the Darknet-53, which is a deep convolutional neural network consisting of 53 layers and used for initial feature extraction. The output of the feature extraction step consists of three different feature maps, where the original input image is down sampled by 32, 16, and 8 times from its original size, respectively. These feature maps are then passed through another 53 fully convolutional layers of the Object Detector module of the YOLOv3 network to produce actual detection kernels. The final YOLOv3 architecture is a 106 layer deep neural network, which produces detections at three different scales (using previously produced feature maps of different sizes) to allow accurate detection of objects with varying size. The tree detection kernels produced at layers 82, 94, and 106 are then combined in a vector with the coordinates of all three detections and corresponding probabilities of the final combined bounding box being a pig.

YOLOv3 is not perfect and will detect bounding boxes without a pig in them (i.e., false positives). False positives (FP) will create extra IDs that are difficult to filter after tracking; thus FP were removed after detection. Frames with FP were identified when more than six pigs were detected, since there were six pigs housed per pen. When this occurred, the six bounding boxes with the highest probability of being a pig were kept, and the extra bounding boxes were removed. This resulted in a deletion of 6,563 detections in the barren environment (out of 3,741,073 detections) and 3,080 detections in the enriched environment (out of 3,733,521 detections). After the first removal of detections, all bounding boxes with a probability of detecting a pig lower than 0.5 were removed to ensure that all random detections were deleted. This resulted in a deletion of another 4,992 detections in the barren environment and 2,680 detections in the enriched environment.

Tracking Method

Simple Online and Real-Time (SORT) was used to track pigs in their home pen (Bewley et al., 2016). The detections produced by YOLOv3 network were used as the input for the SORT tracking algorithm. The performance of SORT is highly dependent on the quality of the initial detection model since SORT has no such functionality itself. The SORT algorithm utilizes the combination of common techniques such as the Hungarian algorithm and Kalman filter for object tracking. The Kalman filter is used to predict future positions of the detected bounding boxes. These predictions serve as a basis for continuous object tracking. This filter uses a two-step approach. In the first prediction step, the Kalman filter estimates the future bounding box along with the possible uncertainties. As soon as the bounding box is known, the estimates are updated in the second step and uncertainties are reduced to enhance the future predictions. The Hungarian algorithm predicts whether an object detected in the current frame is the same as the one detected in the previous adjacent frame. This is used for object re-identification and maintenance of the assigned IDs. The robust re-identification is crucial for

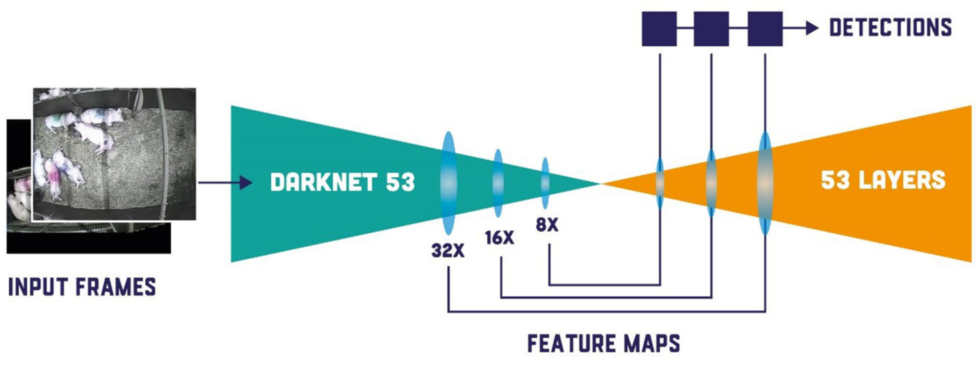

FIGURE 2 | Graphical overview of You Only Look Once v3 (YOLOv3).

continuous and efficient multiple object tracking. The Hungarian algorithm uses different measures to evaluate the consistency of the object detection/identification (e.g., Intersection over Union and/or shape score). The Intersection over Union (IoU) score indicates the overlap between bounding boxes produced by the object detector in one frame and another frame. If the bounding box of the current frame overlaps the bounding box of the previous frame, it will probably be the same object. The shape score is based on the change in shape or size. If there is little change in shape or size, the score increases, guaranteeing re-identification. The Hungarian algorithm and the Kalman filter operate together in SORT implementation. For example, if object A was detected in frame t, and object B is detected in frame t+1, and objects A and B are defined as the same object based on the scores from the Hungarian algorithm, then objects A and B are confirmed being the same object. The Kalman filter could use the location of object B in frame t+1 as a new measurement for object A in frame t to minimize uncertainty and improve the overall score.

Evaluation

Detection results were evaluated by using mean average precision (mAP), intersection over union (IOU), number of false positives (FP) and number of false negatives (FN). mAP is the mean area under the precision-recall curve for all object classes. IOU represents the overlap between two bounding boxes. FP are detections of a pig which is not a pig, where FN are missing detections of a pig.

The tracking algorithm generates more tracks (i.e., part of the video with an assigned ID) than individuals, so each track was manually traced back to the individual that was tracked. Not all tracks could be traced back to a pig, and these tracks are referred to as FP tracks (**Figure 3A**). Occasionally, individuals take over the track of another pig. This is referred to as ID switches (**Figures 3B,C**).

RESULTS

Detection

Figure 4 shows the mean average precision (mAP) and intersection over union (IOU) for all three detection models. In both environments, the mAP was over 99%. The combined detection model reached a mAP of 99.95% in the enriched environment. In both environments, IOU was the lowest with the young detection model. Adding older animals (i.e., combined detection model) improved the IOU in the enriched environment. The old detection model had the highest IOU in the barren environment.

Figure 5 shows the number of FP and FN for all detector models in both environments. The detector trained on young animals found 128 FP in the barren environment where it only found two FP in the enriched environment. FP in the barren environment dropped drastically when older animals were used in or added to the detection model. FN (undetected pigs) decreased in both environments when older animals were used compared to only using younger animals. For both environments, FN dropped even further when young and old animals were combined in the detection model. The combined detection model was used in tracking since it performed best in both environments.

Tracking

In the barren environment, more tracks were identified compared to the enriched environment (**Table 1**). In both environments, approximately one third of the tracks were a FP track. In other words, one-third of the IDs found could not be assigned to a pig. More IDs were switched in the barren environment compared to the enriched environment. FP tracks had a short duration in both environments. On average, the length of FP tracks was 9.9 s in the barren environment and 2.2 s in the enriched environment. When these short FP tracks were excluded, on average individual pigs were tracked for 22.3 min in the barren environment and 57.8 min in the enriched environment.

There was variation between individuals in the performance of the tracking algorithm (**Tables 2**, **3**). In the barren environment the highest number of tracks traced back to one individual was 57, whereas in the enriched environment this was only 23 tracks. The lowest individual average track length was therefore 18 min, where the highest was 138.3 min in the enriched environment.

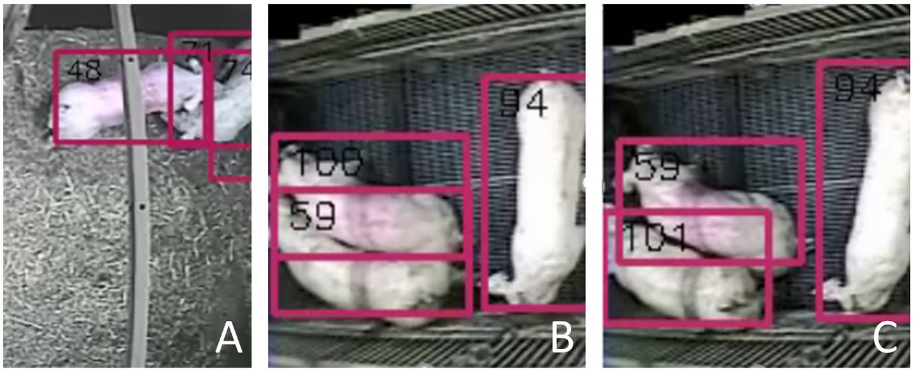

FIGURE 3 | Examples of FP tracks and ID switches: **(A)** An example of a false positive (FP) track, where two pigs are visible, but three bounding boxes are identified. The bounding box most right (nr. 74) is labeled as a FP track since it could not be assigned to a pig; **(B)** The moment just before the ID switch of pig nr. 59; **(C)** Just after the ID switch, where bounding box nr. 59 has moved up to another pig compared to **(B)**. The original pig nr. 59 received a new ID.

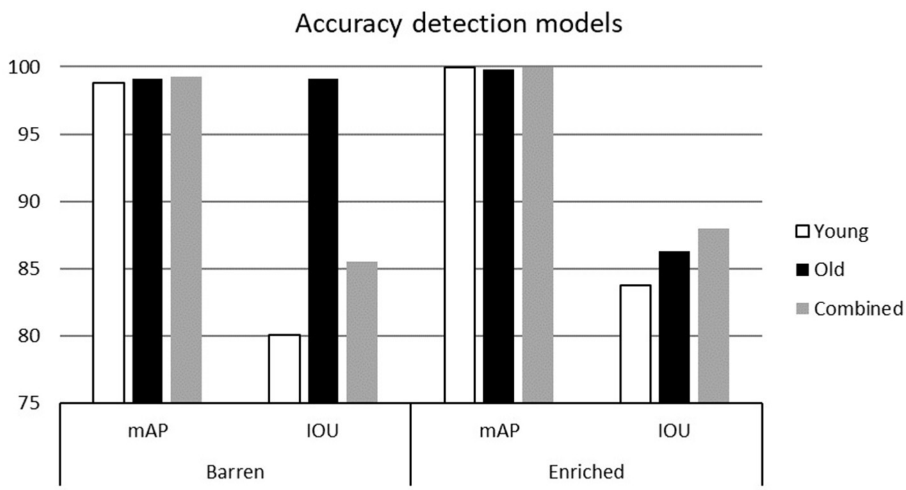

FIGURE 4 | Mean Average Precision (mAP) and Intersection Over Union (IOU) for the barren and enriched environment using the "young," "old," and "combined" detection model.

The average individual track length in the barren environment varied between 7.1 and 41.5 min.

Figures 6, **7** display all tracks per individual including FP tracks. In both environments there was a period between ∼11:24 and 13:48 where all IDs were maintained. In this period, the pigs were mostly lying down. Especially before and after this period of resting most new IDs were assigned to individuals. In these periods the pigs were actively moving around and interacting with pen mates.

DISCUSSION

The aim of this study was to investigate the potential of state-of-the-art CV algorithms using YOLOv3 and SORT for pig detection and tracking for individual activity monitoring in pigs. This study showed the potential of state-of-the-art CV algorithms for individual object detection and tracking. Results showed that individual pigs could be tracked up to 5.3 h in an enriched environment with maintained identity. On average, identity was maintained up to 24.8 min without manual corrections. In tracking-by-detection methods, as used in this study, tracking results are dependent on the performance of the detection method. No literature was found showing an algorithm maintaining identity for longer than 1 minute on average without manually applied marking. The highest average tracking time reported until now was 49.5 s (Cowton et al., 2019). This study outperformed existing literature in maintaining identity in tracking pigs with an average tracking duration of 57.8 min. However, this study used a long video sequence of 7 h, while pigs are known to be active during certain periods of time. This might result in a distorted comparison between studies. However, when the average length of tracks is calculated based on trajectories during active time, the average length of the enriched housed pigs is still between 6.4 and 17.2 min. The average track length of barren housed pigs was lower (3.3–24.6 min), but still higher than found in the literature. The main

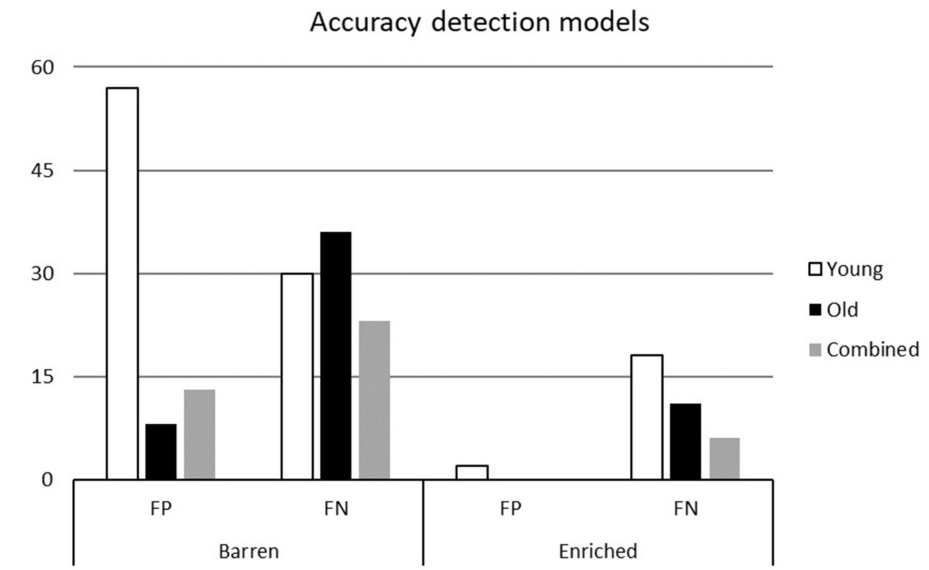

FIGURE 5 | The number of false positives (FP; i.e., tracks not associated with a pig) and false negatives (FN; i.e., undetected pig) for the barren and enriched environment using the "young," "old," and "combined" detection model.

TABLE 1 | Summary of tracking results in barren and enriched environment.

	Barren	Enriched
Number Ids	225	100
False positive track	76	31
Switched Ids	20	4
% tracked of the video	99.3	99.9
Average track without FP[a] (min)	22.3	57.8
Average track with FP[a] (min)	11.3	24.8
Longest track (min)	222.9	315.7

[a]*FP, false positive.*

TABLE 2 | Tracking summary per individual in barren environment.

Barren					
Individual	# tracks	Switches	Total tracked frames	Percentage tracked	Average length (min)
A	20	2	610,719	98.09%	20.4
B	57	4	609,563	97.91%	7.1
C	10	4	622,566	100.00%	41.5
D	21	4	622,028	99.91%	19.7
E	14	4	622,508	99.99%	29.6
F	27	2	620,416	99.65%	15.3

TABLE 3 | Tracking summary per individual in enriched environment.

Enriched					
Individual	# tracks	Switches	Total tracked frames	Percentage tracked	Average length (min)
A	7	0	622,281	99.96%	59.3
B	3	0	622,559	100.00%	138.3
C	23	1	620,568	99.68%	18.0
D	20	2	620,948	99.74%	20.7
E	10	0	622,463	99.99%	41.5
F	6	1	622,387	99.97%	69.2

difference between this study and others is the use of YOLOv3 as a detector.

The proposed tracking algorithm was trained and tested on annotated frames from different ages. Yang et al. (2018) tested their algorithm on different batches within the same pig farm and results were "quite good." They state: "the size of pigs does not matter much." This study, however, proves otherwise. There is a difference in performance between different ages within the same environment (i.e., different size of pigs). Psota et al. (2020) also had a training set that consisted of different pen compositions, angles and ages. They reported that a dataset containing frames from finisher pigs performed better than a dataset containing frames from nursery pigs. This is in line with results presented in the current study, where IOU of the old detection model was higher than the IOU of the young detection model. Another phenomenon was shown in current results: in the enriched environment, the IOU of the combined detection model exceeded the IOU of the young and the old detection model, while in the barren environment the old detection model performed best. This interaction between environment and age could be explained by unoccupied surface in the pen. Enriched housed pigs had twice as much space available than barren housed pigs. In addition, pigs grow rapidly and especially in

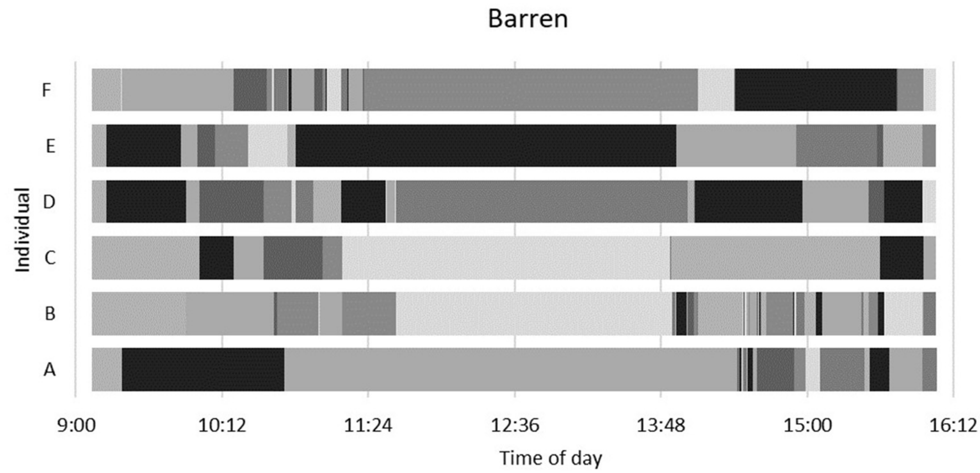

FIGURE 6 | Tracks per individual in the barren environment including false positive tracks corresponding to the time of day.

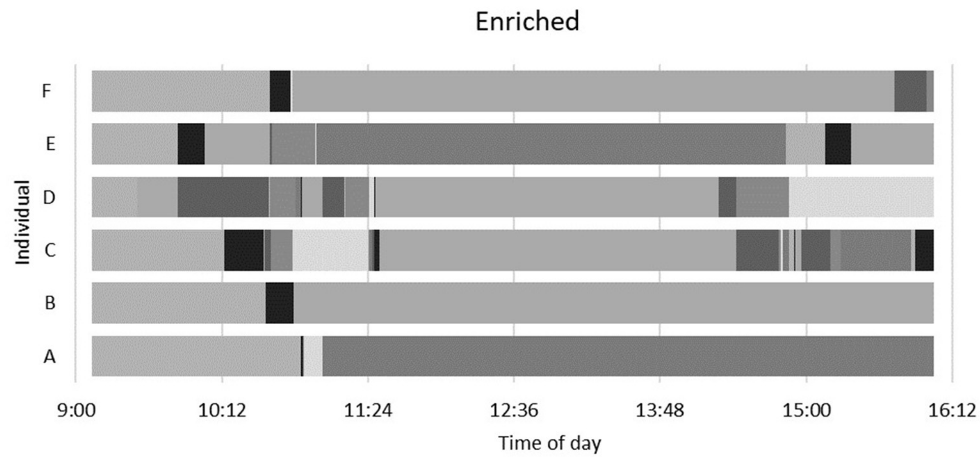

FIGURE 7 | Tracks per individual in the enriched environment including false positive tracks corresponding to the time of day.

the barren environment, pigs are more occluded when growing older. Visually, the frames of the old detection model are more similar to the test frames than the frames of the young detection model (**Figure 8**). Thus, the old detection model fits the test frames the best in the barren environment, and therefore has the best performance. When annotations of younger animals are added, some noise is added in the detections, creating a more robust detection model (higher mAP) with a lower IOU.

Besides the difference in age, there was also a difference in the environment in the current study. The tracking algorithm performed better in the enriched environment rather than in the barren environment. The only difference between the two environments was the use of bedding material and enrichments and the space allowance per pig. The bedding material was not observed to be detected as a pig, so the space allowance is responsible for the difference in performance. The most difficult situations to detect pigs individually is when pigs are touching each other. When in close proximity, IDs can be lost or switched, which could happen more often when there is less space available per pig.

The appearance of pigs (i.e., spots or color marking) appeared to be irrelevant in the performance of the tracking algorithm. Some pigs were colored for identification in the experiment with a saddle-like marking that was prominently visible to the human observer. The tracking algorithm was not affected by the coloring. A pig with a pink marker had the most tracks in the barren environment (**Table 2**; individual C) but was among the pigs with the fewest tracks in the enriched environment (**Table 3**; individual F). Creating more tracks per individual appears to be more strongly related to unfortunate placement of the pig within the pen rather than disturbance by background colors or shadows.

ID switches are a difficult problem in tracking. Not only do you lose the identity, but identities are switched without any

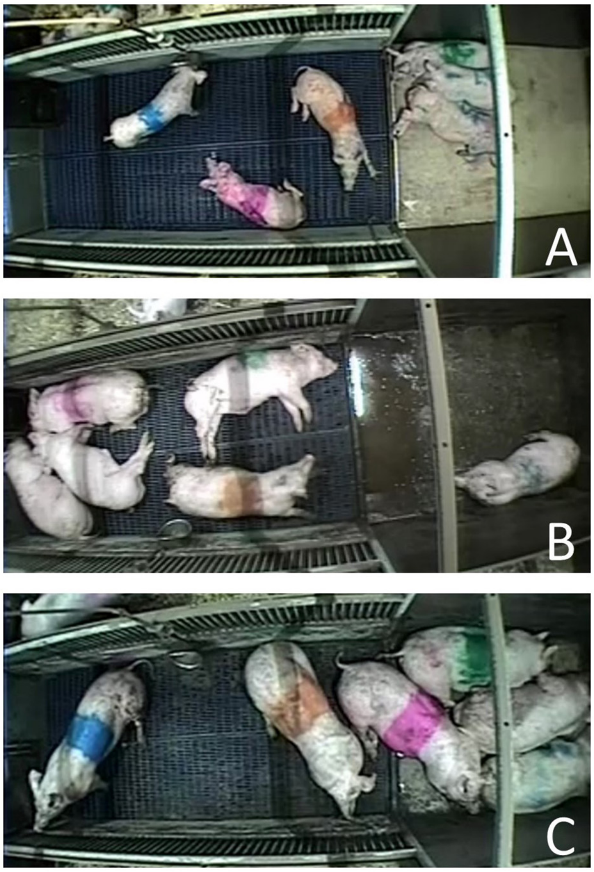

FIGURE 8 | Example frames of young detection model **(A)**, test video **(B)**, and old detection model **(C)**.

visibility in tracking data except when IDs are checked manually. Psota et al. (2020) also reported ID switches. An example showed that despite all animals were detected, only seven out of 13 had the correct ID. We expected that two tracks would exchange their IDs, however, that only happened twice out of 24 switches identified. The other 22 switches showed one individual receiving a new ID number, and the other individual took over the other animal's original ID. These one-sided switches are not well-described in the literature (Li et al., 2009). An advantage of this type of switches is that it is easier to trace back in tracking data since a new ID is created in the process and usually this new ID only has a limited track length. However, it still remains an issue in tracking data. Removing these false positives based on short track length seems a viable way to correct for ID switches.

The algorithm used in this study showed is a first step to measure resilience in future applications. Individual activity or variation in individual activity under stress is a potential indicator of resilience (Cornou and Lundbye-Christensen, 2010; van Dixhoorn et al., 2016; Nordgreen et al., 2018; van der Zande et al., 2020). The algorithm presented estimated bounding boxes and connected them between frames with assigned IDs. When the trajectory is lost, a human observer needs to assign the trajectory to the right ID. Using this algorithm for six pigs, the human observer needs to correct on average the IDs 10 times per hour for enriched housed pigs, and 22 times per hour for barren housed pigs. For a commercial management system, this would still be too labor-intensive, but for research-purposes this is possible. To improve performance further, multiple sensors should be integrated to achieve high accuracy with less labor (Wurtz et al., 2019). To recognize damaging behavior using proposed algorithm is challenging due to the low occurrence of such behavior. Posture estimation could be integrated in proposed algorithm since these behaviors occur regularly. However, for research purposes, this algorithm allows tracking activity of a larger number of individual animals in a non-invasive manner. From location data of every frame, distance moved could be calculated.

CONCLUSIONS

The aim of this study was to investigate the potential of state-of-the-art CV algorithms using YOLOv3 and SORT for pig detection and tracking for individual activity monitoring in pigs. Results showed that individual pigs could be tracked up to 5.3 h in an enriched environment with maintained identity. On average, identity was maintained up to 24.8 min without manual corrections. Using annotations of a combination of younger and older animals had the best performance to detect pigs in both the barren and the enriched environment. The tracking algorithm performed better on pigs housed in an enriched environment compared to pigs in a barren environment, probably due to the lower stocking density. The tracking algorithm presented in this study outperformed other studies published to date. The better performance might be due to the different detection method used, variation in environment, time of day or the size of the training data used. Thus, based on tracking-by-detection algorithm using YOLOv3 and SORT, pigs can be tracked in different environments. The tracks could in future applications be used as an estimate to measure resilience of individual pigs, by recording activity, proximity to other individuals and use of space under varying conditions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Dutch Central Authority for Scientific Procedures on Animals.

AUTHOR CONTRIBUTIONS

TR, LZ, and OG contributed to the conception of the study. LZ and OG developed the tracking algorithm. LZ performed the analysis and wrote the first draft. All authors reviewed and approved the final manuscript.

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The Liquid Diet Composition Affects the Fecal Bacterial Community in Pre-weaning Dairy Calves

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Feeding a liquid diet to the newborn calf has considerable implications for developing the intestinal microbiota, as its composition can shift the population to a highly adapted microbiota. The present work evaluated 15 Holstein calves individually housed and fed one of the three liquid diets: I – whole milk ($n = 5$), II – milk replacer (22.9% CP; 16.2% fat; diluted to 14% solids; $n = 5$) and III – acidified whole milk to pH 4.5 with formic acid ($n = 5$). All animals received 6 L of liquid diet, divided into two meals, being weaned at week 8 of life. Calves also had free access to water and starter concentrate. After weaning, all calves were grouped on pasture, fed with starter concentrate, and hay *ad libitum*. The fecal samples were collected at birth (0) and at weeks 1, 2, 4, 8, and 10 of life. The bacterial community was assessed the through sequencing of the V3-V4 region of the 16S rRNA gene on the Illumina MiSeq platform and analyzed using the DADA2 pipeline. Diversity indices were not affected by the liquid diets, but by age ($P < 0.001$) with weeks 1 and 2 presenting lower diversity, evenness, and richness values. The bacterial community structure was affected by diet, age, and the interaction of these factors ($P < 0.01$). Twenty-eight bacterial phyla were identified in the fecal samples, and the most predominant phyla were Firmicutes (42.35%), Bacteroidota (39.37%), and Proteobacteria (9.36%). The most prevalent genera were *Bacteroides* (10.71%), *Lactobacillus* (8.11%), *Alloprevotella* (6.20%). Over the weeks, different genera were predominant, with some showing significant differences among treatments. The different liquid diets altered the fecal bacterial community during the pre-weaning period. However, differences in the initial colonization due to different liquid diets are alleviated after weaning, when animals share a common environment and solid diet composition.

Keywords: animal nutrition, *Bifidobacterium*, dairy calf, gut health, gut microbiota

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INTRODUCTION

The intestinal microbiota is essential for the stable development of the gastrointestinal tract (GIT) and a healthy immune system (Czarnecki-Maulden, 2008). Nutrition and management, among others, can affect the GIT microbiota of newborn calves (Malmuthuge et al., 2015). Nutrition is the most influential factor in pre-weaned calves' gut bacterial community (Klein-Jöbstl et al., 2014; Guzman et al., 2015). Indeed, the composition of the diet offered to calves affects the gut microbiota structure because it provides different substrates for the growth of bacterial communities (Maslowski and Mackay, 2011; Li et al., 2012; Kasparovska et al., 2016). However, most studies that evaluated the effects of feeding management on the calves' gut microbiota have focused on the impact of a solid diet (Callaway et al., 2010; Shanks et al., 2011; Petri et al., 2013a; Dias et al., 2018), and little information is available on the effect of liquid diet composition on gut microbial composition (Górka et al., 2011; Edrington et al., 2012; Deng et al., 2017).

The main liquid diet offered to pre-weaned dairy calves in Brazilian and Canadian dairy herds is whole milk (Vasseur et al., 2010; dos Santos and Bittar, 2015), while milk replacer is mainly used in USA (United States Department of Agriculture, 2014). Other liquid diets include waste milk, pasteurized waste milk, transitional milk, or acidified milk (milk replacer, waste milk, or whole milk; Vasseur et al., 2010; United States Department of Agriculture, 2014; dos Santos and Bittar, 2015). The liquid diet is commonly offered for 60–90 days after birth, but in some cases, calves are weaned after 90 days.

Whole milk feeding has considerable implications for gut microbiota development and selection for a highly adapted intestinal microbiota dominated by *Bifidobacterium* (Kelly et al., 2016). These bacteria have probiotics effects on gut development and the prevention of dysbiosis (Hidalgo-Cantabrana et al., 2018). Probiotic is defined as a living microorganism that promotes a benefit to host's health (Hill et al., 2014). Various milk constituents, such as oligosaccharides and glycoconjugates, selectively enrich this type of microorganism (Pacheco et al., 2015). In humans, newborns fed on breast milk compared to infant formula have a gut microbiota with a profound relationship to neonatal enterocyte genes that influence host protection and development (Schwartz et al., 2012). Also, formula-fed babies have more significant colonization by *Clostridium spp*. and particularly *C. difficile* (Penders et al., 2005, 2006; Vael and Desager, 2009), microorganisms associated with various diseases, including diarrhea (Dial, 2004; Poutanen, 2004; Lees et al., 2020).

In many dairy herds, especially those that have *ad libitum* feeding, the liquid diet is acidified with organic acids up to pH 4.2–4.5 to maintain its microbiological quality throughout the day (Todd et al., 2017). This type of liquid diet is an alternative for feeding dairy calves and has been studied as a simple and low-cost method (Yanar et al., 2006). Acidification can modulate the gut microbiota, retarding growth, or eliminate pathogenic microorganisms (Richard et al., 1988; Jaster et al., 1990), sensitive to lower pH values. Besides, acidification can favor the growth of beneficial microorganisms, such as *Lactobacillus*, *Bifidobacterium,* and *Faecalibacterium* (Deng et al., 2017), that will compete for nutrients with pathogenic microorganisms.

The use of commercial formulas (milk replacer) can be an option to replace whole milk, either for economic reasons, for consistency in the liquid diet composition, or for the opportunity to increase the solids content and consequently the performance of calves. Many studies have shown that milk replacers may be suitable to replace whole milk, ensuring animal performance, as long as they have an adequate composition as to the sources and levels of nutrients (Cooper and Watson, 2013; Bittar et al., 2018; Badman et al., 2019; Bai et al., 2020). The various ingredients used in the milk replacer composition, such as dairy products or vegetable origin products (Bittar et al., 2018), can affect the gastrointestinal microbiome (Badman et al., 2019).

Some studies have used fecal sampling to study the gut microbial community in dairy calves (Uyeno et al., 2010; Oikonomou et al., 2013; Badman et al., 2019), especially for ease of sampling, and because it is a non-invasive method, allowing repeated sampling (Claesson et al., 2017). However, due to the microbial community's difference between the mucosa and digesta, and differences along the intestinal tract, the fecal microbial community should not be used to represent the entire gut microbiota (Tang et al., 2020). It is more accepted as a proxy for the distal gut microbiota (Claesson et al., 2017).

This study aimed to compare the fecal bacterial community of calves fed different liquid diets (whole milk, acidified whole milk, and milk replacer) in the pre- and post-weaning phase. We have investigated the fecal bacterial community of dairy calves using 16s rRNA amplicon sequencing as a non-invasive proxy for the intestinal bacterial community in fifteen young dairy calves. We hypothesized that feeding different liquid diets can cause fecal bacterial community changes during the pre-weaning period, and that these changes are persistent after weaning.

MATERIALS AND METHODS

Animals, Facilities, and Feeding System

This study was conducted at the calf facilities of the Department of Animal Science, Luiz de Queiroz College of Agriculture, University of São Paulo, located in Piracicaba – São Paulo, Brazil. All animal procedures followed the guidelines recommended by the Animal Care and Use Committee (Protocol no. 2018.5.586.11.7).

This study was part of a performance study with 36 newborn Holstein calves (male calves, birth weight of 32.84 ± 1.54 kg; and females, 28.22 ± 0.81 kg), blocked according to sex, age, and birth weight and evaluated in a randomized block design in the pre-weaning period for performance and health. Previous results were published in the abstract by Coelho et al. (2020a). For the present study, 15 calves were assigned to 5 blocks so that each block had 3 calves with similar sex, age, and BW at the beginning of the experiment. Then, three male blocks (birth weight 28.52 ± 0.91 kg), and two female blocks (birth weight 35.89 ± 2.24 kg), were used to assess the impact of different liquid diets on the fecal bacterial community.

All animals were fed 10% of birth-weight of high-quality colostrum (>50 g IgG/L) within the first 6 h of life (Godden, 2008). All calves had serum protein above 5.5 g/dL at 48 h of life, as recommended by Elsohaby et al. (2019). From the second day of life, each calf within each block was fed one of the three evaluated liquid diets: 1 - Whole milk (WM); 2 - Acidified whole milk (AWM); and 3 - Milk replacer (MR; Sprayfo Azul, Sloten from Brazil Ltda, SP, Brazil) diluted to 14% solids. The composition of the MR and whole milk are described in **Table 1**.

The total volume of whole milk was collected daily in the milking parlor and divided into two portions, one part was immediately refrigerated, and the other was acidified. The pH

TABLE 1 | Chemical composition of the starter, milk replacer and whole milk.

Composition	Starter concentrate	Milk replacer	Whole milk
Dry matter, %	89.96	96.49	–
Ash, % DM	6.93	8.73	–
Crude protein, % DM	22.66	22.90	2.28*
Crude fat, % DM	2.92	16.20	3.89*
NDF, % DM	16.47	1.13	–
NFC, % DM	51.02	51.04	–
Lactose, %	–	–	4.45
Total solids, %	–	–	12.58

NDF, Neutral detergent fiber; NFC, Non-fiber carbohydrate (NFC) was calculated according to the equation: NFC = 100 – (CP + EE + NDFcp + ash).
**Not in the dry matter, but estimated from total solids (g/100 g milk).*

was measured with a pH-meter (Tecnal, SP, Brazil) during the acidification process, which was stopped when pH reached 4.5. The acidification was done with the milk at 5°C to avoid the formation of protein clots, with the addition of formic acid (formic acid 85%, Dinâmica Química Contemporânea Ltda, SP, Brazil). The milk was acidified at least 12 h before feeding and kept at room temperature. Because the pH dropped during storage, it was corrected back to 4.5 by adding whole milk before being supplied to the calves. All liquid diets were heated to 38–40°C before feeding. Liquid diets were supplied from 2 d of life till weaning, 3 L of liquid diet was individually offered twice daily (7 and 17 h). Calves were trained from 2 d of life to drink milk from an open bucket. At 57 d of age, the weaning process was initiated in the morning by reducing the total daily liquid diet supply by 1 L every day until complete weaning at 62 d.

Calves Housing

Immediately after birth, the calves were housed in individual suspended cages (113 × 140 cm) with sawdust beds in a ventilated barn, where they remained until 15 d of life. At 2 d, the calves had free access to water and starter concentrate in open buckets located in the cage's front. From 16 d until the end of weaning at 62 d, the calves were individually housed outdoors in a wood shelter with free access to water and starter concentrate. The commercial starter concentrate (Bezerra Ag Milk Agroceres Multimix Animal Nutrition Ltda., Rio Claro, SP, Brazil) was offered daily *ad libitum*, always after supplying the liquid diet. The composition of starter concentrate is described in **Table 1**.

After weaning, calves were grouped on pasture, with free access to water, the same starter concentrate, and hay *ad libitum*. The animals were followed up to 70 days of age when the study ended.

Animal Health and Measurement

The individual consumption of the liquid diet and the starter concentrate, was measured daily. Calves were weighed at birth and weekly until week 8 on a mechanical scale (ICS-300, Coimma Ltda., Dracena, SP, Brazil), always before morning feeding. Average daily gain (ADG) and feed efficiency (kg of BW gain/kg of total DMI) were calculated for the pre-weaning period (0–56 d).

The fecal score was monitored daily, as described by Larson et al. (1977), based on the fluidity of feces: (1) normal and firm; (2) soft; (3) aqueous; (4) fluid. Diarrhea was considered when the calves had a fecal score ≥ of 3 for more than 1 day. Calves with a score ≥ of 3 received oral rehydration solution[1] in a volume of 8% of body weight, 2 h after morning feeding, with a bottle until the fecal score returned to normal. Calves' rectal temperature was measured daily using a digital thermometer, and fever was considered when the calf had more than 39.4°C. Health problems were monitored and treated according to veterinary recommendations.

Evaluation of Bacterial Community

Fecal Samples Collections

Fecal samples were collected at day 0 (±1 h after birth, before colostrum feeding) and at days 7 (S1), 14 (S2), 28 (S4), 56 (S8, weaning), and 70 (S10, post-weaning). The samples were collected manually with gloves, directly from the animals' rectum, and the gloves were discarded at each collection to avoid cross-contamination among samples. About 2 g of feces were collected, placed in sterile tubes, and immediately frozen at −20°C.

DNA Extraction, Library Preparation, and Sequencing

DNA extraction from fecal samples was performed with the QIAamp® Fast DNA Stool Minikit extraction (Qiagen, Hilden, Germany), following the modifications suggested by Yu and Morrison (2004). The quality of the DNA samples was evaluated by electrophoresis on 0.8% agarose gel and concentrations were quantified with a spectrophotometer (NanoDrop® ND-2000; Thermo Fisher Scientific, Wilmington, DE, USA).

The libraries were prepared following Illumina's recommendations. The primers used for locus-specific amplification of bacteria flank the V4 region. Overhang sequence of adapters is included in locus-specific primers. Illumina adapter sequences, which were hybridized with the immobilized sequences on the sequencing sheet, were:

Forward overhang: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[locus-specific sequence]

Reverse overhang: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[locus-specific sequence]

The first PCR was performed for locus-specific amplification. Then, AMPure XP beads were used to purify the PCR reaction, and the size of the fragments generated in the PCR reaction was evaluated by agarose gel electrophoresis. The second PCR was performed to connect the barcodes of the Nextera XT kit, and new steps for purifying the PCR and validating the libraries were performed. Subsequently, the libraries were quantified so that all samples/libraries were joined in an equimolar manner in a single pool.

A heterogeneous control, the phage phi-X, was combined with the amplicon pool to introduce complexity to the sequencing. Finally, the libraries and phi-X have been denatured to allow

[1]Composition: 1 L of warm water, 1 g of potassium chloride, 80 g of dextrose, 4 g of sodium bicarbonate, and 5 g of sodium chloride.

sequencing. Sequencing was performed in Illumina Miseq system (Illumina, San Diego, CA, USA) and produced readings were 2 × 250 bp. All raw DNA sequence reads were deposited in NCBI's Sequence Read Archive under BioProject PRJNA639165, submission SUB7576848.

Bioinformatic Analyses

The data were analyzed as a previously published pipeline (Callahan et al., 2016b), using a set of packages implemented in the R language[2] (R Core Team) and available through the BioConductor project (Gentleman et al., 2004; Huber et al., 2015).

First, multiplexed readings were assigned to biological samples. The DADA2 program (Callahan et al., 2016a), an open-source package implemented in the R language, was used to model and correct amplicon errors without building OTUs. Callahan et al. (2016a) show that DADA2 identified more real variants in several simulated communities and produced fewer spurious sequences than other methods. The DADA2 package has a complete pipeline implemented to transform the sequencer fastq files into sequences of inferred, dismembered samples and without chimeras.

The filtering of fastq files was performed to cut the PCR primers' sequences and filter the 3′ends of the readings due to the quality decay ($Q < 30$), but maintaining the overlap for later joining of the readings and reassembly of the fragment of the V4 region. The DADA2 algorithm uses a parametric error model, and each set of amplicon data has a different set of error rates.

After the initial processing of the sequencing data by DADA2, taxonomies were assigned to each ASV (Amplicon Sequencing Variants) using an implementation of the DADA2 program of the naive Bayesian classifier method for this purpose (Wang et al., 2007). The assignTaxonomy function takes as input a set of sequences (ASVs) to be classified, and a set of training of reference sequences with known and assigned taxonomies. The SILVA database was used as a reference (Glöckner et al., 2017).

The taxonomic classifications generated by DADA2, and their quantifications, were imported into the phyloseq program (McMurdie and Holmes, 2013), also implemented in R. The α and β diversity analyses were performed with the phyloseq package, as described in Callahan et al. (2016b). For the β-diversity analysis, a multivariate permutational analysis of variance (PERMANOVA) was performed, using weighted UniFrac distances, testing the treatment effect, week, and interaction. ASVs that have not been classified until the family level were filtered, and ASVs marked as being the same species have been clustered. After applying these filters, the tables of gross abundance and relative abundance counts were obtained.

Then, the taxonomic counts in the phyloseq object were imported into the edgeR package (Robinson et al., 2010) to normalize the sizes of each sample's libraries (Robinson and Oshlack, 2010), subsequently the counts were transformed to the base 2 logarithms of the counts per million (log CPM) of each sample (voom transformation; Law et al., 2014). These transformations allow the linear models implemented in the limma package (Ritchie et al., 2015) to analyze differential abundance. Finally, after adjusting the linear model with limma, the differential taxonomic abundance was tested for each contrast (pair of treatments) with moderate *t*-tests (Smyth, 2004).

[2] https://www.R-project.org/

Statistical Analysis

The experimental design was a randomized block design, with the animals allocated in the blocks according to birth weight, age, and sex. Before model construction, the normality of residues for all variables was verified by the Shapiro -Wilk test using the PROC UNIVARIATE procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Average daily gain (ADG), starter concentrate intake, consumption of the liquid diet, feed efficiency, and rectal temperature were analyzed as time repeated measures, using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC), according to model: $Yijk = \mu + Ti + Bj + Eij + Wk + TWik + Eijk$, where, $Yijk$ = variable response; μ = general mean; Ti = fixed effect of treatment (liquid diets); Bj = random block effect; Eij = residual error A; Wk = fixed age effect (weeks); $TWik$ = fixed effect of interaction treatment and age; $Eijk$ = residual error B. The covariance structures "compound symmetry, heterogeneous compound symmetry, autoregressive, heterogeneous autoregressive, unstructured, banded, variance components, toeplitz, antidependence and heterogeneous toeplitz" were tested and defined according to the lowest value obtained for "Akaike's Information Criterion corrected" (AICC).

The variable cumulative days per calf affected by diarrhea and days in fever were analyzed by "Restricted Maximum Likelihood" ANOVA for a randomized complete block design (RCBD) using PROC MIXED. As the days with diarrhea was not normally distributed, its analysis was performed based on log-transformed data. If significance was detected by ANOVA F test, the Student's *T*-test was assessed for the comparison among the means. The fixed variables were evaluated using the following statistical model: $Yji = \mu + Ti + bj + eij$, where μ = general mean; Ti = fixed effect of treatment (liquid diets); bj = random block effect; and eij = residual error.

Kaplan-Meier survival curves were plotted for cumulative diarrhea events for WM, MR, and AWM groups using SAS 9.4 (SAS Inst. Inc., Cary, NC). The endpoint of interest was survival time, which was defined as the time to first diarrhea event (fecal score ≥ 3; 1 to 4-point scale) or the end of the study in days. For this, a data set was organized containing the variables treatment (WM, MR, and AWM), time (representing the disease-free survival time), and status (censoring indicator, with the value 1 indicating an event time and the value 0 indicating a censored time). The STRATA command of SAS 9.4 (SAS Inst. Inc., Cary, NC) was used to test the null hypothesis between different survival curves according to the log-rank (Mantel-Cox) test. Mantel Haenszel hazard ratio and its confidence interval were performed using the Cox proportional hazard model using the slope of the survival curve. It was conducted to compare the rate of a diarrhea event occurring between the treatments over time. Additionally, Cox proportional hazard model was fitted to compare the rate of diarrhea incidence up to 15 days after

TABLE 2 | Performance, intake and health from birth to weaning of calves fed with different liquid diets.

Item	Treatment			SEM	*p*-value		
	AWM	MR	WM		LD[1]	A[2]	LD × A[3]
BW (kg)							
At birth	32.68	32.32	33.82	3.04	0.171	–	–
At weaning	68.16[a]	61.04[b]	69.72[a]	4.89	0.073	–	–
ADG (kg/d)	0.599[ab]	0.528[b]	0.673[a]	0.04	0.025	<0.001	0.011
Starter intake (g)[4]	227.27	285.74	201.52	68.75	0.529	<0.001	0.761
Liquid diet intake (L)[4]	5.77[b]	5.88[ab]	5.98[a]	0.04	0.097	<0.001	0.043
Feed efficiency[4]	0.60[b]	0.51[c]	0.70[a]	0.03	<0.001	0.019	0.050
Rectal temperature (°C)	38.33	38.43	38.44	0.09	0.205	<0.001	0.926
Days with fever[5]	0.60	2.40	2.00	0.64	0.165	–	–
Diarrhea (days)[6,7,8]	8.04[ab]	9.83[a]	4.92[b]	1.63	0.040	–	–

[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$. AWM, Acidified whole milk; MR, Milk Replacer; WM, Whole milk. [1]LD, liquid diet. [2]A, age. [3]LD × A, Interaction between liquid diet and age. [4]d 1 – 56; [5]number of animals with diarrhea: AWM (n = 3); MR (n =5); WM (n = 4); [6]Based on Student's T-test at $P < 0.05$. [7]Statistical analysis was based on natural log-transformed data but means are back-transformed to the original scale; [8]number of animals with fever: AWM (n = 4); MR (n = 5); WM (n = 4).

enrollment date. For all the analyses, differences detected at $P \leq 0.05$ were considered significant.

RESULTS

Feed Intake and Health of Calves

Body weight at birth did not differ among treatments. However, at weaning AWM and WM-fed animals tended to have higher BW when compared to those fed MR (**Table 2**; $P = 0.073$). The starter concentrate intake increased with age (**Figure 1B**; $P < 0.001$) but was not affected by treatment or by the treatment interaction with age (**Table 2**). The rectal temperature was higher in the first 4 weeks of life (**Figure 1D**; $P < 0.001$), but the values were not indicative of fever, and it was also not affected by the treatment or interaction of both factors (**Table 2**). The number of days with fever was also not affected by the treatments (**Table 2**). The consumption of the liquid diet was affected by age and interaction of age and treatments, and tended to be affected by treatment (**Table 2**; $P < 0.001$; $P = 0.043$; $P = 0.097$, respectively). At week 1, intake was higher for WM and MR than AWM (**Figure 1A**; $P = 0.033$), and this effect tended to be observed at week 3 as well (**Figure 1A**; $P = 0.053$). The ADG was affected by treatments, age and interaction of both factors (**Table 2**; $P = 0.025$; $P < 0.001$; $P = 0.011$). In the pre-weaning period, the ADG was variable (**Figure 1C**), at week 2 and 3 WM animals tended to present greater gain than MR and AWM, respectively ($P = 0.069$; $P = 0.052$). At week 4, WM had a greater gain than AWM and MR ($P < 0.001$), and at weeks 5 and 6, MR-calves had the smallest gain ($P = 0.014$; $P = 0.038$, respectively). Feed efficiency was affected by treatments, age and interaction of both factors (**Table 2**; $P < 0.001$; $P = 0.019$; $P = 0.050$). The efficiency was also variable during the pre-weaning period (**Figure 1E**). At weeks 2, 3, and 4, WM-fed calves had the greatest feed efficiency ($P = 0.031$; $P = 0.047$; $P < 0.001$, respectively) and MR-fed calves tended to be less efficient than AWM calves at weeks 5 and 6 ($P = 0.072$; $P = 0.054$, respectively).

Number of cumulative days affected by diarrhea was higher for calves fed MR compared to WM, whereas AWM-calves were similar to the two other groups ($P = 0.040$; **Table 2**). However, the Log-Rank model's diarrhea incidence during the experimental period did not differ among treatments (**Table 3**). The median days for 50% of the animals in each group to be diagnosed with diarrhea showed no difference among treatments (**Figure 2**). However, more MR calves tended to be diagnosed with diarrhea in the first 15 days ($P = 0.09$; **Table 4**), as compared to the WM calves.

Bacterial Community

Sequencing information (Number of raw reads, number of quality-filtered reads, Number ASVs identified) is shown in **Supplementary Tables 1, 2**.

The microbial profile analysis was performed using data from the amplicon sequence of the 16S rRNA gene. **Table 5** shows α-diversity indices. The indices were not affected by different liquid diets. However, all indices were affected by age ($P < 0.001$), but there was no interaction between the liquid diet and age (**Table 5**).

Samples collected at birth (0) had greater diversity (Shannon) and richness (Chao1). Weeks 1 and 2 had less diversity (Shannon and Simpson) and evenness (Pielou), and week 1 had less richness (Chao1; **Figure 3**).

β-diversity was affected by the liquid diet ($P = 0.001$), the age of the animals ($P = 0.001$), and also by the interaction of these factors ($P = 0.03$). While there were dissimilarities in the pre-weaning phase, weaned calves' fecal bacterial community presented similarities in structure (**Figure 4**).

Twenty-eight bacterial phyla were identified in the fecal samples. In general, the predominant phyla were Firmicutes (42.35%), Bacteroidota (39.37%), Proteobacteria (9.36%), Fusobacteriota (4.08%), and Actinobacteriota (3.02%), corresponding to 98.18% of total (**Supplementary Table 3**). Abundance of these phyla over the weeks is shown in **Figure 5A**. Proteobacteria

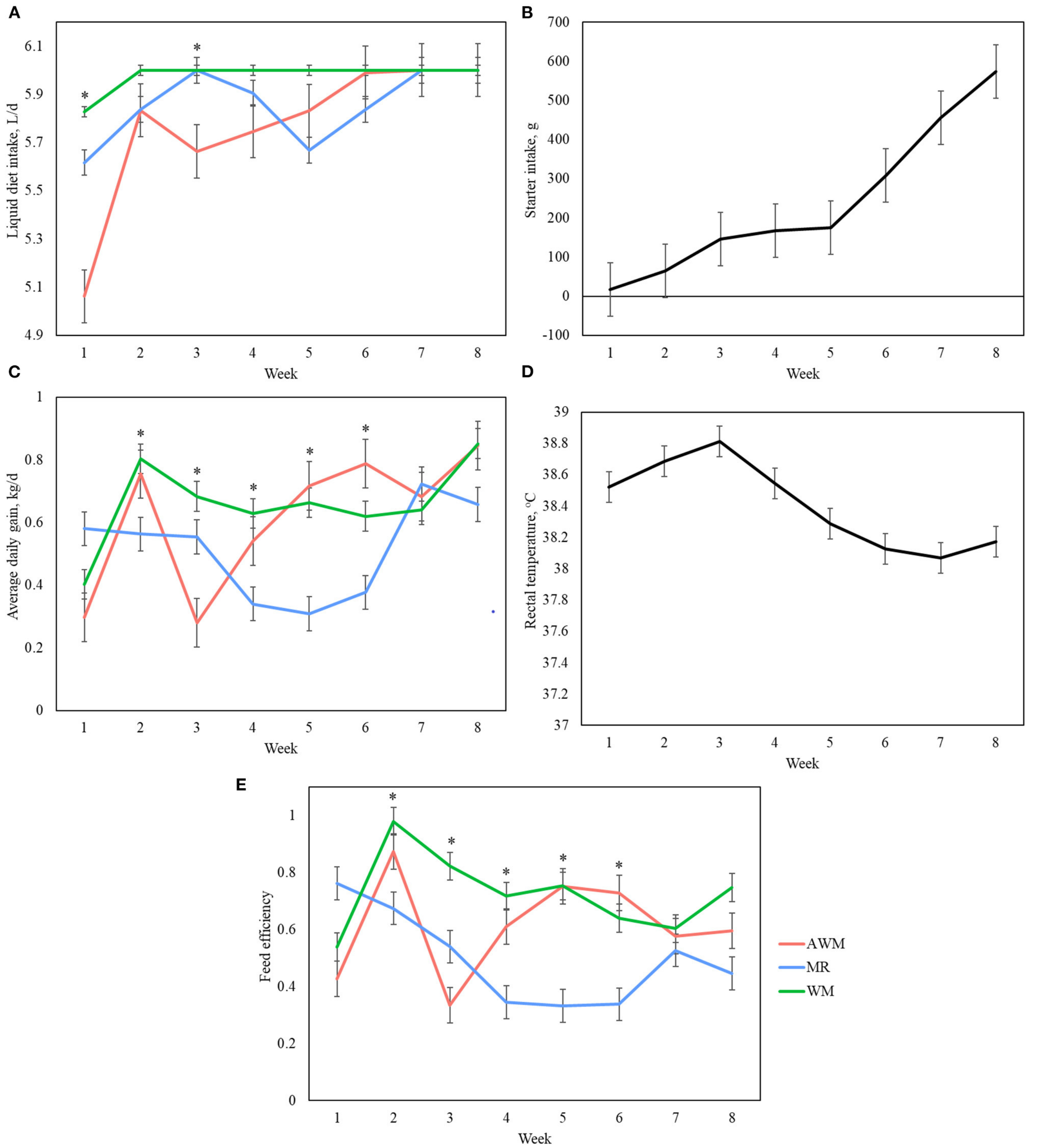

FIGURE 1 | Liquid diet **(A)** starter intake **(B)** average daily gain **(C)** rectal temperature **(D)** and feed efficiency **(E)** of dairy calves fed different liquid diets. AWM, Acidified whole milk; MR, Milk replacer; WM, Whole Milk. *Denotes difference among treatments in the respective week.

decreased at week 1. Bacteroidota was the most abundant at birth. Firmicutes had the greatest abundance until week 4, when it was surpassed at week 8 by the phylum Bacteroidota.

Of these 28 phyla, 559 bacterial genera were identified. Among these genera, the most prevalent were *Bacteroides* (10.71%), *Lactobacillus* (8.11%), *Alloprevotella* (6.20%), *Escherichia/Shigella* (5.21%), and *Faecalibacterium* (5.07%) (**Figure 6**).

TABLE 3 | Log-rank (Mantel-Cox) and Mantel Haenszel hazard ratio comparison between the cumulative incidence of diarrhea from birth to weaning in calves fed with different liquid diets.

	Log-rank χ^2	*P*-value	Survival curve comparison	Hazard ratio	95% CI		*P*-value
					Lower (HR)	Upper (HR)	
Incidence of diarrhea	2.48	0.288	WM vs. MR	2.23	0.72	11.1	0.130
			WM vs. AWM	1.61	0.40	6.48	0.510
			MR vs. AWM	0.57	0.15	2.18	0.410

AWM, Acidified whole milk; MR, Milk Replacer; WM, Whole milk.

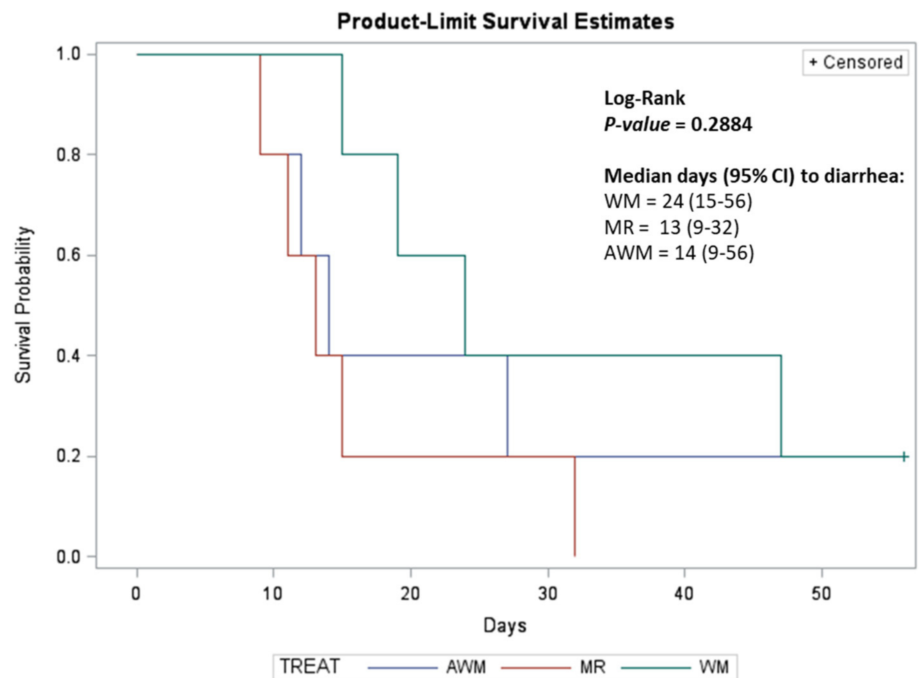

FIGURE 2 | Median days for 50% of animals to be diagnosed with diarrhea in each group of dairy calves fed different liquid diets. AWM, Acidified whole milk; MR, Milk replacer; WM, Whole Milk.

TABLE 4 | Incidence of diarrhea up to 15 days relative to birth date in calves fed with different liquid diets.

Item	Treatment		
	AWM	MR	WM
Incidence of diarrhea, %	20.0	80.0	60.0
Hazard ratio	Baseline	6.87 (0.76–62.4)	4.73 (0.49–46.0)
P-value	–	0.09	0.18

AWM, Acidified whole milk; MR, Milk Replacer; WM, Whole milk.

However, the nine most abundant genera exhibited a distinct abundance profile over time (**Figure 5B**). At birth, the bacterial community was dominated by the genus *Bacteroides* (10.17%). At week 1, however, *Lactobacillus* (22.82%) and *Escherichia/Shigella* (18.06%) overlapped *Bacteroides* (13.65%) and were the most abundant genera, reaching their highest values. After week 1, the abundance of *Escherichia/Shigella* and *Lactobacillus* decreased considerably until week 10 (0.15 and 0.01%, respectively). Even though its abundance decreased, compared to the previous week, *Lactobacillus* remained the most abundant genus in week 2, followed by *Bacteroides* and *Fusobacterium* (15.29, 13.71, and 10.29%, respectively). At week 4, there was a predominance of the genera *Alloprevotella*, *Faecalibacterium,* and *Fusobacterium* (10.00, 8.33, and 6.02%, respectively). At week 8, *Alloprevotella* reached its highest abundance value (14.71%), being predominant at this time, and was followed in abundance by *Bacteroides* and *Prevotella* (12.24 and 6.81%, respectively). At week 10, there was a decline in abundance values for these three genera. However, *Bacteroides* remained the most abundant genus (8.94%), followed by *UCG-005* and *Rikenellaceae RC9 gut group* (8.68 and 5.21%, respectively), which reached their highest abundance values at this point. Data for all genera over the weeks are in **Supplementary Table 4**.

All taxonomic abundance differential data are reported in **Supplementary Table 5**. **Figure 7** shows 15 bacterial genera in a relative abundance of ≥1% on a heat-map. At week 0 and

TABLE 5 | Fecal microbial diversity of calves fed with different liquid diets.

Indices	Diet			SEM	*p*-value		
	AWM	WM	MR		LD[1]	A[2]	LD × A[3]
Shannon	2.51	2.65	2.43	0.07	0.117	<0.001	0.756
Simpson	0.84	0.86	0.82	0.01	0.176	<0.001	0.334
Chao1	62.46	62.98	58.78	3.22	0.586	<0.001	0.733
Pielou	0.63	0.66	0.62	0.01	0.122	<0.001	0.466

[1]*LD, liquid diet.* [2]*A, age.* [3]*LDxA, Interaction between liquid diet and age. AWM, Acidified whole milk; MR, Milk Replacer; WM, Whole milk.*

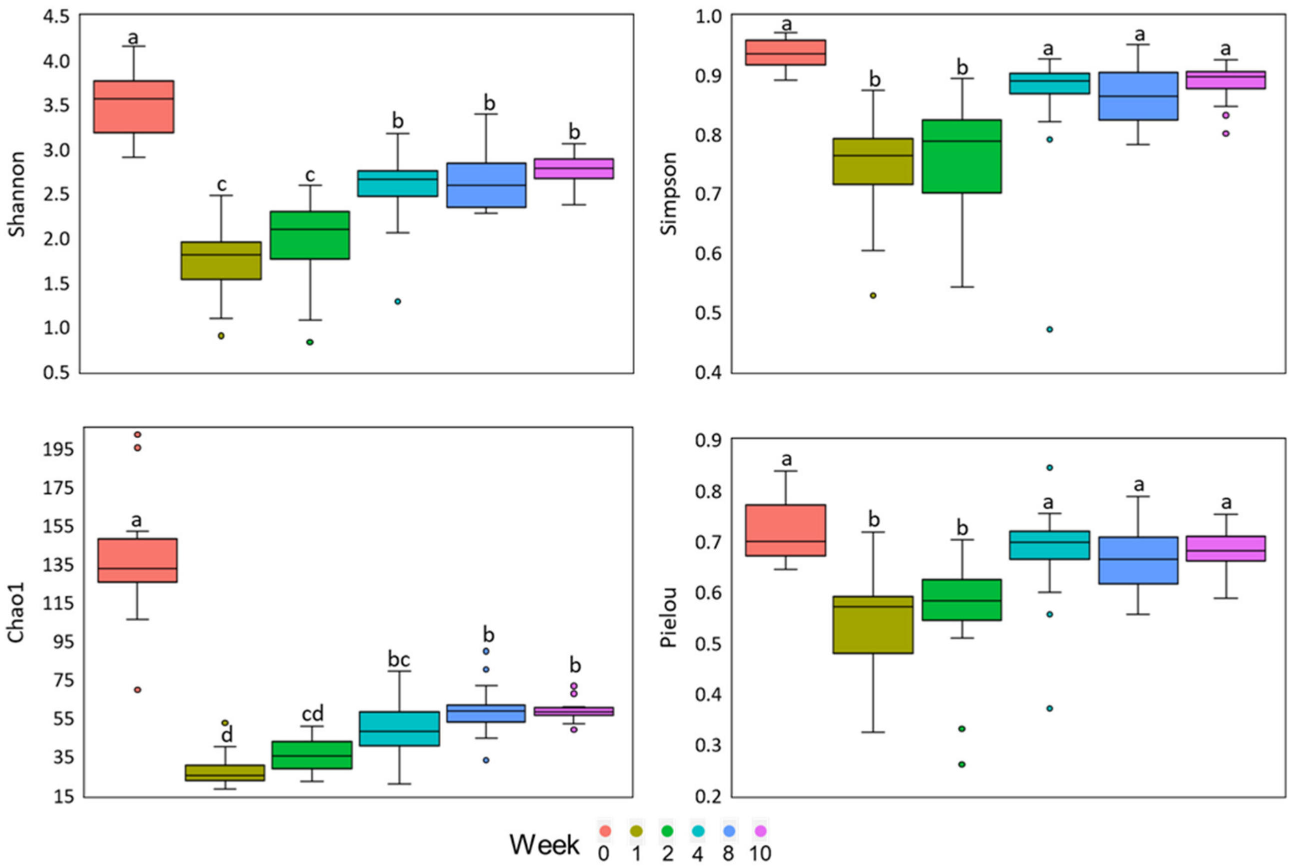

FIGURE 3 | α-diversity indices in fecal samples from dairy calves fed different liquid diets. Data are visualized as box-plots showing the median and the interquartile (midspread) range (boxes containing 50% of all values), the whiskers (representing the 25 and 75 percentiles) and the extreme data points. Letters above boxes indicate significant differences at $P > 0.05$.

week 10, no genus differed among treatments. Weeks 1 and 4 showed the most significant number of differences among treatments. *Alloprevotella* was the most abundant at week 8 (**Figure 6**), and there was no difference among treatments. The differences were evident when *Alloprevotella* had low abundance (weeks 1 and 2). *Bacteroides* had higher, while *Bifidobacterium* had lower abundance in WM than MR at weeks 1 and 4. At week 2, *Bifidobacterium* was lower with AWM. *Butyricicoccus* was higher at week 1 for AWM and WM. *Collinsella* was more abundant at weeks 2, 4, and 8 for WM compared to AWM. *Faecalibacterium* had lower abundance at weeks 1 and 2 in MR-fed calves. *Fusobacterium* was less abundant at weeks 1, 2, 4, and 8 in AWM-fed calves compared to MR. *Lactobacillus* was abundant in WM over the weeks. *Parabacteroides*, *Phascolarctobacterium*, *Prevotella*, *Rikenellaceae RC9 gut group,* and *UCG-005* were less abundant throughout the studied weeks, but these genera were more abundant for WM compared to MR-fed calves.

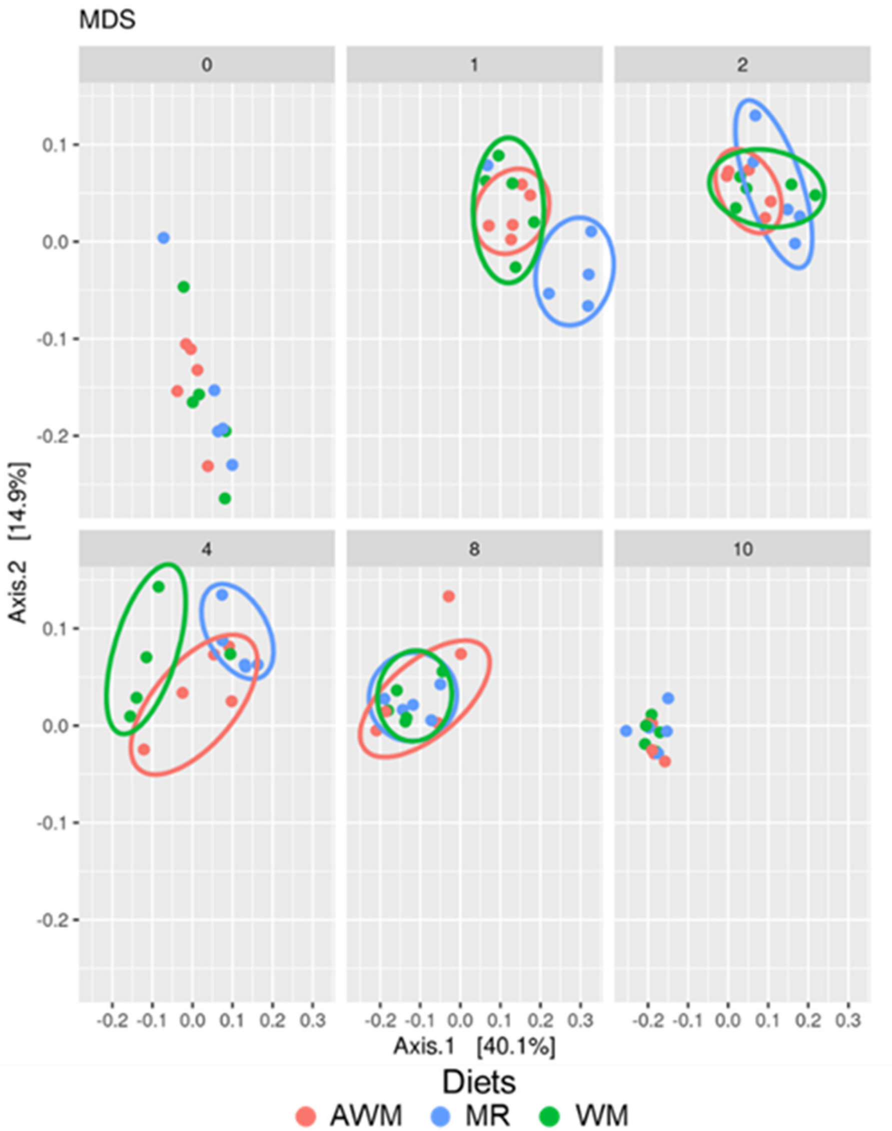

FIGURE 4 | Effect of the interaction of liquid diets with the different ages of the dairy calves on the structure of the microbial community in fecal samples in dairy calves fed different liquid diets. Multidimensional scaling (MDS) showing the weighted UniFrac distance metric. AWM, Acidified whole milk; MR, Milk Replacer; WM, Whole milk.

DISCUSSION

Composition of the Fecal Bacterial Community

In the pre-weaning period, the liquid diet is the primary source of energy and protein. When reaching the small intestine, it serves as a substrate for the growth of microorganisms (Górka et al., 2011). Characterizing the gut microbiota in the pre-weaning phase is of great importance to understand host-microbiome interactions (Badman et al., 2019). In our study, the fecal bacterial community was affected by different liquid diets. It is interesting to note that each diet promoted a greater abundance of specific microorganisms throughout the pre-weaning period, which have directly affected animals' performance and health.

Whole milk, especially unpasteurized milk, in human nutrition has been described in several studies as capable of improving intestinal health (Fagnani et al., 2019; Butler et al., 2020), as it promotes the growth of probiotic microorganisms, as *Lactobactillus*, *Faecalibacterium,* and *Bifidobacterium* (Hill et al., 2014). Recent studies have shown that the intake of unpasteurized whole milk increased the abundance of *Lactobacillus* in the human gut microbiota (Butler et al., 2020). In our study, the consumption of WM was constant in the pre-weaning period, and higher at week 1 than AWM-fed calves. This consumption may have stimulated the genus *Lactobacillus* in these animals compared to those fed with AWM. Probably, this initial stimulus remained until week 4, when consumption did not differ anymore among treatments, as the genus *Lactobacillus* remained

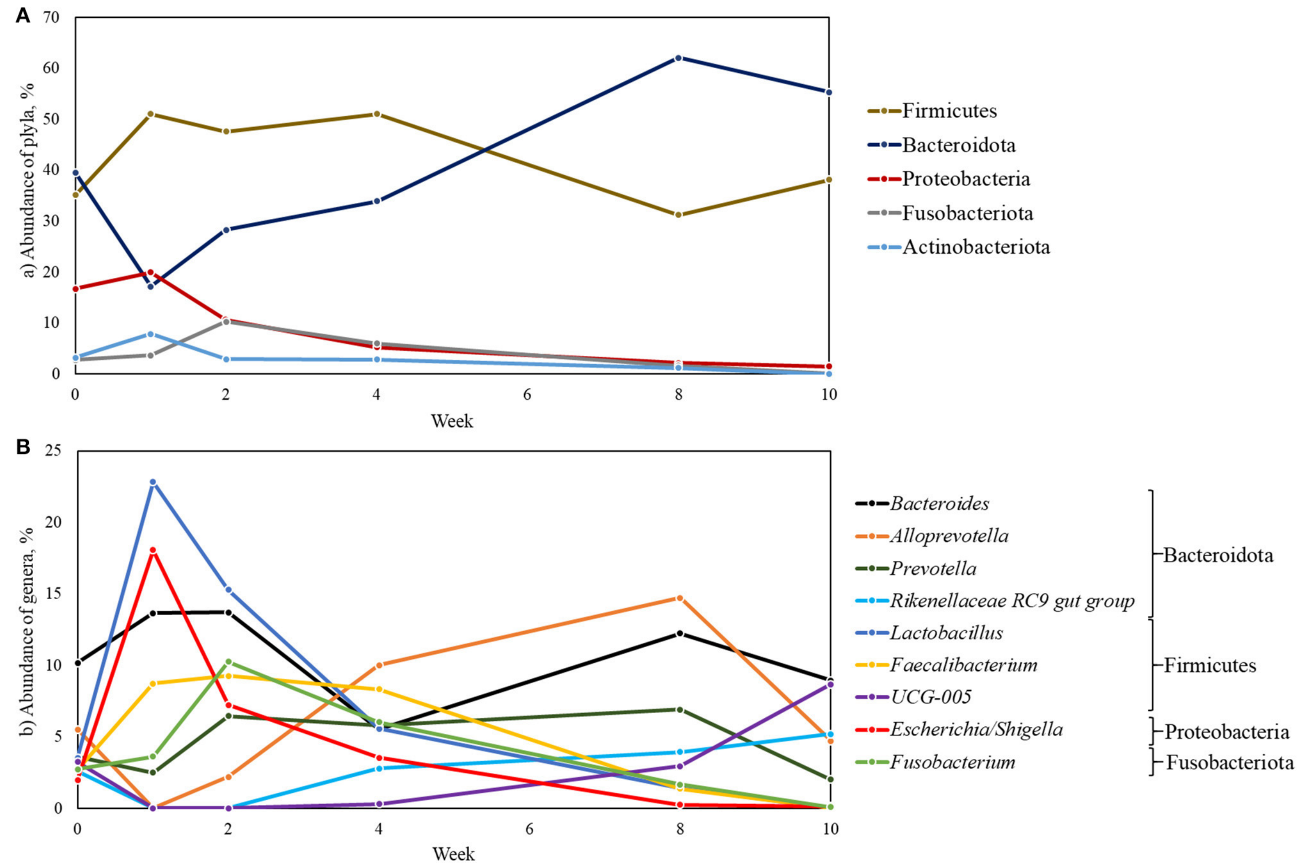

FIGURE 5 | Relative abundance of bacterial phyla **(A)** and genera **(B)** in fecal samples during pre- and post-weaning in dairy calves fed different liquid diets.

more abundant with MR compared to AWM. *Lactobacillus* spp. have been associated with minor infections and diarrheal disorders, in addition to stimulating the mucosal immune system (Abe et al., 1995; Macfarlane et al., 2007). It is interesting to note that the genus *Lactobacillus* was also present in this period, being the most prevalent in the first 2 weeks of age, probably helping to minimize and control diarrhea. The higher initial prevalence of another beneficial bacteria in feces, *Faecalibacterium* spp., was associated with a lower incidence of diarrhea in the first 4 weeks of life, and increased average daily gain in calves (Oikonomou et al., 2013). Similarly, in our study, calves fed WM showed a greater abundance of *Faecalibacterium* at week 1 and a lower incidence of diarrhea, and consequently higher ADG and feed efficiency. Probably, WM feeding provides the necessary substrate for the growth of *Faecalibacterium,* like acetate (Duncan et al., 2002). Unfortunately, we did not analyze short-chain fatty acids in fecal samples to discuss this point.

Milk acidification can promote the same benefits as whole milk, in addition to making the environment unfavorable for the pathogenic bacteria growth that are sensitive to the lower pH (Deng et al., 2017; Coelho et al., 2020b). Another benefit includes the modulation of the digesta' pH (Coelho et al., 2020b), which possibly can benefit the growth of other beneficial microorganisms. Similar to our findings, Deng et al. (2017) observed a greater abundance of beneficial bacteria in the gut of calves fed with waste milk acidified with formic acid. Although this author did not analyze the fecal score, they did investigate the expression of intestinal mucosa genes, and suggested an improvement in general health conditions. Besides in our work, milk acidification increased the age for the first case of diarrhea when compared to WM (15 vs. 9 days). Yanar et al. (2006) also found similar results when acidifying milk replacer with formic acid. Although use of formic acid on acidification process was acceptable, Zou et al. (2017) observed higher inflammation scores in the jejunum and ileum. In general, studies with acidified milk have shown beneficial results for the calves' health as shown in our results on the bacterial community.

Other bacterial genera are described as commensal and beneficial to the gut environment, such as *Alloprevotella*, *Parabacteroides,* and *Phascolarctobacterium* (production of succinate and acetate, lower inflammatory activity; Sakamoto and Benno, 2006; Watanabe et al., 2012; Li et al., 2018) or *Anaerovibrio* and *Butyricicoccus* (improvement of the intestinal barrier; Eeckhaut et al., 2013; Chen et al., 2019). These bacteria were more abundant at weeks 1 and 2 in samples collected from animals fed WM and AWM. The abundance of these genera may

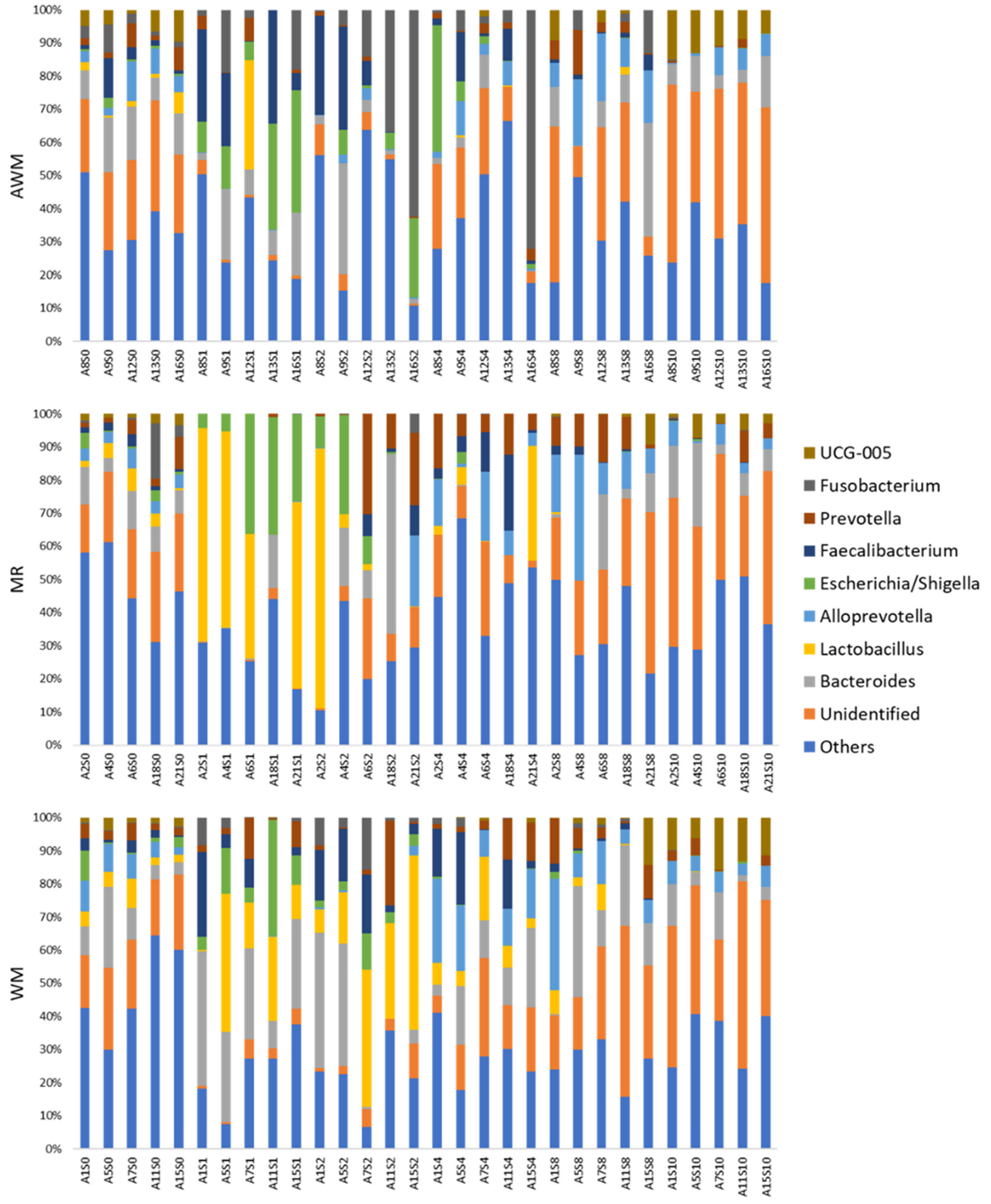

FIGURE 6 | Bacterial relative abundance for the genus level in fecal samples in dairy calves fed different liquid diets. Each bar represents the identification of the analyzed sample: Axx-Sy, where Axx is the animal, Sy, the week in which the collection was performed. AWM, Acidified milk; MR, Milk Replacer; WM, Whole milk.

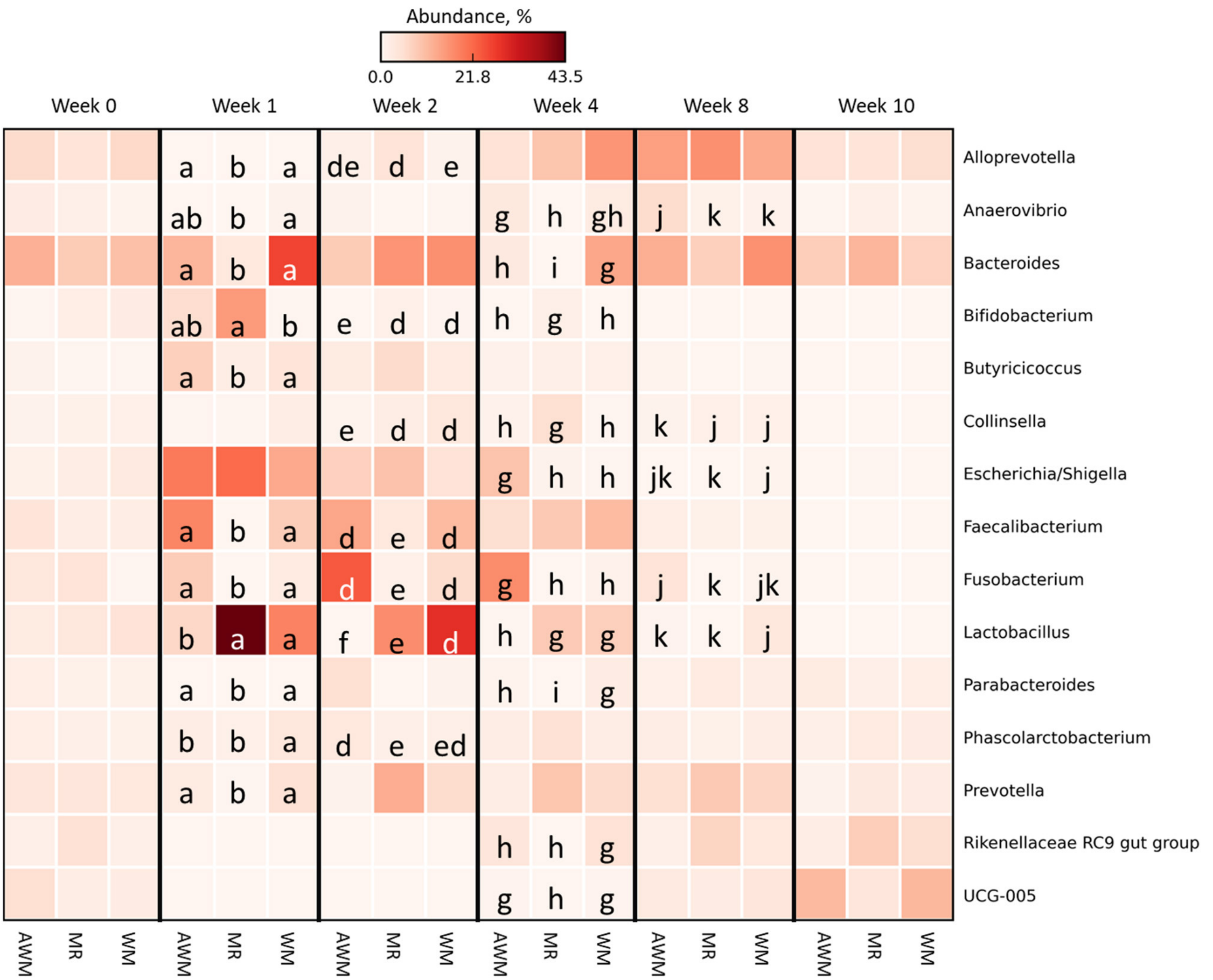

FIGURE 7 | Differential abundance in most abundant bacterial genera in fecal samples of dairy calves fed different liquid diets. Data are visualized as heat-map. Comparisons are among treatments within week. Means followed by the same letter are not significantly different by the *t*-test ($P > 0.05$). Genera rows without letters were not significantly different. AWM, Acidified milk; MR, Milk Replacer; WM, Whole milk.

indicate the modulating effect of these liquid diets on the gut bacterial community.

The composition of MR can alter the gut microbiota (Badman et al., 2019). Some studies report that MR may contain oligosaccharides that act as feed components with high bioactive potential, which can help establishing a beneficial gut microbiota (Aldredge et al., 2013; Badman et al., 2019). Besides, the presence of vegetable oils in MR can affect the bacterial community. The MR in the present study contained coconut oil and palm oil.[3] Recent studies suggest that coconut oil may have a beneficial role in modulating the human bacterial community, increasing *Lactobacillus* and *Bifidobacterium*'s population (Djurasevic et al., 2018; Rolinec et al., 2020), as observed in our study. However, palm oil may not have the same beneficial effects as coconut oil (Mancini et al., 2015). Palmitic acid, present in palm oil, affects the integrity of the intestinal epithelium, causing an unbalanced immune response and stimulating the production of inflammatory cytokines, favoring a pro-inflammatory bowel condition (Ghezzal et al., 2020). This condition may be related to the greater abundance of *Collinsella* at weeks 2, 4, and 8. This genus is associated with pro-inflammatory dysbiosis (Candela et al., 2016; Astbury et al., 2020), increasing intestinal permeability, reducing expression of tight junctions in enterocytes, and stimulating gut leakage (Chen et al., 2016). The loss in intestinal integrity has probably favored diarrhea in MR-fed calves leading to lower ADG, efficiency and BW at weaning compared to WM-fed calves.

Other observations regarding the bacterial community were common to the different liquid diets. We have observed in our data that the abundance of the genera *Lactobacillus*,

[3]Trouw Nutrition: Personal Communication.

Bifidobacterium, and *Faecalibacteirum* decreased with age. Despite their effect on gut development, they were overcome by competition overtime with fiber-degrading bacteria (Dill-McFarland et al., 2017), such as the genera *Fibrobacter* and *Ruminococcus* (**Supplementary Table 4**). The presence of carbohydrate fermenting bacteria, such as *Bacteroides* during pre-weaning, suggests an increased ability to use complex carbohydrates from the starter including cellulose, hemicellulose, resistant starch, and xylans, which reach the large intestine (Dias et al., 2017; Zhang et al., 2017). Although the AWM and WM-fed calves had greater abundance of *Bacteroides* than the calves MR-fed, this did not impact the starter concentrate intake.

Before week 10 of life, there was an increase in *Prevotella* genus' abundance probably due to the increasing starter concentrate intake. This genus has a wide range of metabolic capacities (Petri et al., 2013b; Rubino et al., 2017), as it can use soluble carbohydrates, pectins, proteins, and hemicellulose (Huws et al., 2016). In studies targeting the ruminal bacterial community, *Prevotella* was shown to be the predominant genus. However, although increasing with time, abundance of *Prevotella* in our study remained low, confirming the work of Lourenco et al. (2020) in beef calves, which was probably due to low consumption of a solid diet and competition with other genera. Indeed, starting at week 10, the abundance of *Prevotella* started to drop, probably due to the increase in abundance of *Fibrobacter* and *Ruminococcus* and the change from individual to group-housing. In a study with chimpanzees, Amaral et al. (2017) also observed a decrease in the *Prevotella* genus after the animals had been housed collectively.

α and β-Diversity of the Fecal Bacterial Community

The GIT of newborn calves was traditionally considered sterile at birth and quickly colonized by a diverse microbial population (Mayer et al., 2012). Recent studies have indicated the presence of microorganisms in the meconium of newborn calves (Alipour et al., 2018; Elolimy et al., 2019), and the fetal GIT (Guzman et al., 2020). In our study, we also found a significant bacterial community in the meconium, which may indicate microbial colonization during the fetal period. However, most microorganisms present in meconium were not found at 7 d of life. The greater α-diversity at birth (week 0) suggests that most of the microorganisms at this point are transient, and perhaps they may have a role to play in the initial colonization after birth. According to Fischer et al. (2018), these initial microorganisms can interact uniquely with the host, leading to a high individual bacterial community variation.

The decrease in the α-diversity between weeks 1 and 2 may be a consequence of the development of microorganisms adapted to the extra-uterine environment and, mainly, to colostrum and the liquid diet. The increase in α-diversity has been related to calves' age in the pre-weaning period, as observed by Badman et al. (2019) and Oikonomou et al. (2013). Lower α-diversity in this period can also be associated to higher incidence of neonatal diarrhea, as observed in other studies (Oikonomou et al., 2013; Xie et al., 2013; Nakamura et al., 2017; Zeineldin et al., 2018). Diarrhea is a gut disorder associated with dysbiosis in the bacterial community, and its higher incidence was indicated by the highest average of the fecal score between weeks 1 and 2 (Coelho et al., 2020b). When comparing the bacterial community and the diarrhea data, we can associate this disorder to the genera *Escherichia*/*Shigella* and *Fusobacterium*, which were more abundant in weeks 1 and 2. The genus *Escherichia*/*Shigella* includes the enterotoxigenic *Escherichia coli* species, which are responsible for most cases of neonatal diarrhea in calves (Rigobelo et al., 2006; Izzo et al., 2011). *Fusobacterium* species appear abundant in dysbiosis conditions (Huh and Roh, 2020), and is associated with inflammatory bowel diseases (Ohkusa, 2003). Excessive growth of *E. coli* can be limited by lactic acid-producing bacteria, such as *Lactobacillus* spp. (Ripamonti et al., 2013).

This subsequent increase in α-diversity will likely assist the development of GIT and the following transition from a liquid to an exclusively solid diet. In general, there is a shift in the microbiota to that of adult animals, as the animal increases the starter concentrate intake, which develops the rumen and prepares for weaning (Lallès, 2012). Besides, analysis of β-diversity indicated similarities among treatments, mainly at week 10 when calves were already weaned, group-housed, and under the same management. Cohabitation allows hosts to share microorganisms (Song et al., 2013; Wang et al., 2016; Diao et al., 2019), decreasing dissimilarity among animals.

The different liquid diets altered the fecal bacterial community during the pre-weaning period. The supply of whole milk was associated with a higher abundance of beneficial bacteria and consequently higher performance. The supply of acidified whole milk can be an alternative for the pre-weaning period, considering the gut microbiota, even if the calves are less efficient than those fed with whole milk. The supply of milk replacer must be carefully evaluated. However, differences in the initial colonization due to different liquid diets are alleviated after weaning, when animals share a common environment and solid diet composition.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, BioProject PRJNA639165, submission SUB7576848.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Research Ethics Committee of "Luiz de Queiroz" College of Agriculture, University of São Paulo (Protocol No. 2018.5.586.11.7).

AUTHOR CONTRIBUTIONS

CB and GV conceived and designed the experiment, wrote, reviewed, and edited the manuscript. CB and LC provided experimental and laboratorial resources. MC and AT managed the calves. GV, MC, and AT conducted sample collection. GV performed the lab analyses. HM conducted data analyses and interpretation of results. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2021.649468/full#supplementary-material

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frontiers in Animal Science

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Simple but Complex—A Laying Hen Study as Proof of Concept of a Novel Method for Cognitive Enrichment and Research

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Enrichment can reduce stress and stereotypic behavior and therefore enhance captive animal welfare. In cognitive enrichment, cognitive tasks engage and challenge the animals' natural behavioral repertoire and provide mental stimulation. Enrichment with similarities to "puzzle boxes" in cognitive research is widespread in zoos but rarely applied in commercial farming, as it requires costly time and effort. Here, we introduce a flexible method for cognitive enrichment and research. The test battery apparatus (TBA) is a configurable cubic box with frames for interchangeable test panels, each holding a problem-solving task that must be solved for a food reward. As a proof of concept, we report observations and first results from two groups of laying hens (*Gallus gallus* forma domestica; 52 birds in total) to show the TBA's feasibility in commercial farming and to investigate the animals' spontaneous interaction with four test panels. While we could not reliably identify individuals, we found the majority of the hens highly motivated to engage with the device. At least five individuals in each group were successful and there was a significant gradient of success rates across the four panels. As the implementation and maintenance required little time and effort, the TBA is promising as a cognitive enrichment device in farm settings. Its potentially limitless configurations allow diverse opportunities for cognitive and behavioral engagement in the long term. While further studies will be crucial to validate welfare effects and problem-solving tasks, the TBA is simple in its application but complex in its possibilities.

Keywords: cognitive enrichment, problem solving, laying hen, *Gallus gallus*, chicken, proof of concept, test battery apparatus

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INTRODUCTION

Enhancing the welfare of captive animals is an important societal concern and research topic. Animal welfare does not only include physical health but essential behavioral and mental needs, stimulating environmental challenges and agency (Broom, 1986; Dawkins, 1990; Špinka and Wemelsfelder, 2011). In commercial farming, practical issues such as costs and feasibility complicate animal welfare improvements (Webster, 2001). Legally, animal husbandry must be appropriate to the animals' "physiological and ethological needs in accordance with established experience and scientific knowledge" (Council of Europe, 1976), implying the necessity to adapt to novel scientific developments. One approach to meet animals' ethological needs is environmental enrichment (Newberry, 1995; Shepherdson, 1998) that has been linked to positive welfare effects such as mental stimulation, improved fine motor skills and, if applied correctly, reduced negative stress and stereotypic behavior in numerous species (Carlstead and Shepherdson, 2000; Swaisgood and Shepherdson, 2005).

A subset of environmental enrichment is cognitive enrichment targeting the animals' cognitive skills. As defined by Clark (2011), cognitive enrichment "(1) engages evolved cognitive skills by providing opportunities to solve problems and control some aspect of the environment, and (2) is correlated to one or more validated measures of wellbeing" (p. 6). The need for an intrinsic or extrinsic reward was later added as a third condition (Clark, 2017). One important aspect of any effective enrichment is variation and change. Enrichment devices that are installed once and permanently only reward the same behavior do not offer long-term challenges needed for positive eustress and less boredom (Meehan and Mench, 2007; Selye, 2013).

A meta-analysis of publications from 1985 to 2004 found variable environmental and cognitive enrichment to be absent or at least very rare in farm animals (de Azevedo et al., 2007), most likely due to feasibility and costs. Enrichment in commercial poultry farming commonly consists of inflexible items such as pecking stones, alfalfa bales, strings or straw (Schreiter et al., 2019). In recent years, the appraisal and discussion of cognitive skills of farm animals has received more attention, implying an important need for more mental stimulation for livestock in farm settings (Nawroth et al., 2019). Operant conditioning has been utilized as cognitive enrichment (Meyer et al., 2010) and while its direct impact on animal welfare is difficult to correlate and quantify, there are some promising studies showing positive effects on some species of farm animals (e.g., pigs: Ernst et al., 2005; Manteuffel et al., 2009; goats: Langbein et al., 2009; Kalbe and Puppe, 2010; Zebunke et al., 2013). Chickens (*Gallus gallus* forma domestica) are an underrepresented species in the increased research interest in farm animal cognition of recent years (Nawroth et al., 2019) and numerous welfare problems are known in this species (for a review, see Janczak and Riber, 2015). While cognitive enrichment in poultry is virtually non-existent, a number of cognitive skills have been shown in chickens that principally allow, if not require, it to be implemented (Krause et al., 2006; Smith and Johnson, 2012, Tahamtani et al., 2015; Dudde et al., 2018; Garnham and Løvlie, 2018).

In contrast to farm settings, cognitive enrichment is more common in zoos (e.g., Clark et al., 2013; Yamanashi et al., 2016). Additionally, providing zoo animals with hidden food that can be obtained by overcoming obstacles or opening containers is widespread and, while not being validated scientifically, can involve cognitive challenges. Interestingly, typical tests of problem-solving abilities in animal cognition research share some similarities with this kind of enrichment: "puzzle boxes" with special food rewards that can be accessed by solving a cognitive problem (e.g., Benson-Amram et al., 2016; Borrego and Gaines, 2016). However, puzzle boxes with a single solution are not variable and flexible enough for long-term application as cognitive enrichment. A more complex and variable version of a puzzle box is the multi-access-box (MAB) (Auersperg et al., 2011; Huebner and Fichtel, 2015; Johnson-Ulrich et al., 2018; Williams et al., 2021). Instead of only one solution to obtain food there are several solutions in a MAB that can be discovered, explored and learned stepwise. In this paradigm, results can be discussed with regard to problem solving, cognitive flexibility and (repeated) innovation (Auersperg et al., 2012). Importantly, the MAB approach allows diverse behavioral engagement with a single relatively small apparatus—an aspect of interest for low-maintenance enrichment devices. Another approach to diversity and flexibility in cognitive enrichment is a modular maze idea of the "Gorilla Game Lab" (Gray et al., 2018; Clark et al., 2019). Different problem-solving modules can be connected and flexibly arranged to create a changing enrichment device that is challenging in the long term. However, in contrast to the MAB approach, this device has to be elaborately assembled outside of the animals' enclosure with high demands of space and effort.

Here, we introduce a novel device called the "Test Battery Apparatus" (TBA) for cognitive enrichment and animal cognition research. Inspired by the MAB, the TBA is a cubic box baited with a food reward that allows multiple different problem-solving tasks at the same time to single animals or groups. But similarly to the modular maze approach, it is also flexible and expandable in a way that it supports, in principal, an almost limitless number of additional tasks that can easily be exchanged. The TBA is closed on the bottom and with transparent top and front surfaces (**Figure 1**). The remaining three sides of the cube (S1–S3) are open frames into which test panels containing problem-solving tasks can be inserted and easily relocated and exchanged for other panels. The tasks can be specifically adapted to the animals' natural and ecologically-relevant behaviors in species-specific ways. The TBA approach aims toward bolstering cognitive enrichment in farm animals and the development of a low-cost and low-effort method for a variety of species while potentially improving animal welfare in a diverse and complex manner over the long term.

The variable configuration of the TBA with its interchangeable test panels has the potential for a test battery or "mini test battery" approach (see Shaw and Schmelz, 2017) in a single basic apparatus. It is therefore not only of interest as a cognitive enrichment device but also as a behavioral research apparatus, potentially targeting a variety of topics: Problem-solving abilities can be investigated on a group level and between species and on an individual level within species. Other potential tests include novel object tests, commonly applied to measure shyness/boldness in animals (e.g., Wilson et al., 1994; Coleman and Wilson, 1998; Stöwe et al., 2006; Herrmann et al., 2007), a detour test, commonly applied to measure inhibitory control in animals (e.g., MacLean et al., 2014; Nawroth et al., 2016), a persistence test with an unsolvable problem (e.g., Rao et al., 2018) and several repeated measures of activity and exploration—all in one apparatus. The open-frame design of the TBA is not limited to a specific research topic but can be creatively expanded and adopted in various ways for future research.

In this study, we applied the TBA as potential cognitive enrichment to two separate groups of untrained and naïve

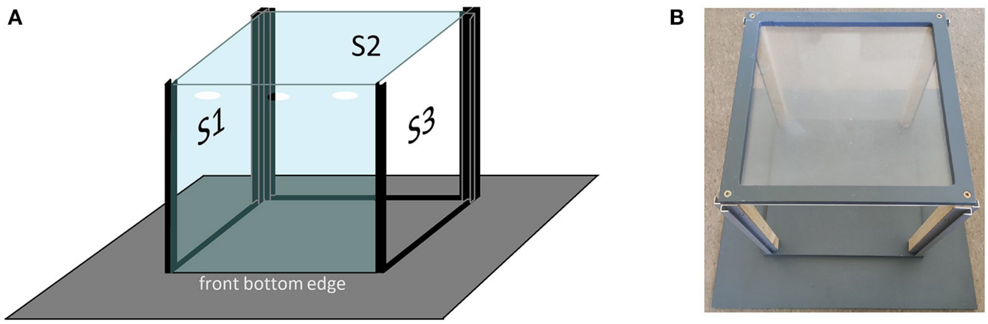

FIGURE 1 | (A) Schematic drawing of the Test Battery Apparatus (TBA) on its platform without inserted panels in the open frames of the sides S1 (left), S2 (back), and S3 (right). **(B)** Photo of TBA from the opposite side. The open frame of S2 can be seen on the front, the frames of S1 and S3 are on the right and left side, respectively. The far side and top are permanently covered with transparent acrylic glass.

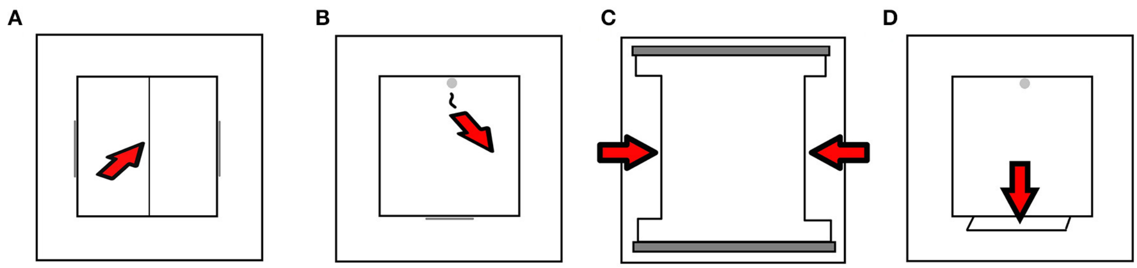

FIGURE 2 | The four test panels that can be inserted in the frames of the TBA: **(A)** swing panel, **(B)** string panel (invisible magnet behind door in light gray), **(C)** slide panel, **(D)** step panel (invisible magnet behind door in light gray). Arrows illustrate the solution.

laying hens as a proof of concept. We aimed to (1) investigate the interaction of the groups with four test panels, presenting different problem-solving tasks adapted to the birds' behavioral repertoire (see **Figure 2**) and (2) show the basic feasibility in a commercial farm setting. We expected the laying hens to spontaneously engage with the TBA in general and the test panels in particular whenever it was baited with a food reward and to be able to successfully solve the tasks of these panels at differing success rates. Furthermore, we expected the use of the TBA to be simple from the humans' point of view and therefore applicable to a farm setting with minimal disturbance of the daily routines.

METHODS

Animals

We tested two groups of producing laying hens that were available for testing at the Friedrich-Loeffler-Institute for Animal Welfare and Animal Husbandry in Celle, Germany, between October 2019 and April 2020. In total, 52 laying hens (∼20 months old at the beginning of the study) were allocated in the two separate groups that were housed next to each other and tested sequentially. These animals had individually participated in a previous learning study with no methodological similarities to the problem-solving tasks presented here (Dudde et al., in prep.). They were kept in standard litter floor system pens of about 11 m^2 with wood-shavings, perches, and a group nest. About 2.5 × 3 m of each pen were an unobstructed open ground area.

Apparatus and Procedure

The TBA box was made of PVC (thickness 1 cm), transparent acrylic glass (thickness 0.4 cm), and wooden posts (thickness 2 cm) on the four corners (see **Figure 1B**). Its dimensions were 30 × 30 × 30 cm attached to a 50 × 50 cm heavy platform to preclude it from being moved or toppled over by the animals. The TBA with open frames, that is without inserted panels in sides S1–S3 (as depicted in **Figure 1**), was placed in the middle of the ground floor area of each of the pens of the laying hen groups continuously (4 and 2 months, respectively). A handful of wheat grains, a food reward laying hens have been shown to be motivated to work for in previous studies (see Dudde et al., 2018), was put inside the open TBA at irregular intervals (ranging from daily to ca. weekly) to habituate the animals to the box in the beginning and then establish it as a part of their pen and a feeding location. The TBA remained in place inside each of the pens throughout the course of the experiment and was roughly cleaned before each test session and thoroughly cleaned and disinfected when it was moved to the other group.

In the test sessions, one of four different test panels was inserted into the frame of side S2, while the other two frames, S1 and S3, always held plain opaque panels with no opening. The food reward inside the TBA could therefore only be accessed by solving the problem of the respective test panel on the side S2 in these sessions. All test panels were designed to allow the hens to use behaviors within their natural behavioral repertoire but in a specific way not encountered before (pulling string with beak, pushing door forward or sideways with beak or body, pressing pedal downwards with beak or feet). In particular, the four test panels were:

Swing Panel

An opening in the panel (20 × 20 cm) was blocked by an opaque two-winged door with hinges on both sides. To access the food, the door had to be pushed inside (**Figure 2A**).

String Panel

An opening in the panel (20 × 20 cm) was blocked by an opaque hatch of the same size that was attached with a hinge on the bottom and held in place by a magnet on the top back side. On the top front a short string with a knot was attached to the hatch. To access the food, the string had to be pulled to disengage the magnet, causing the hatch to fall open to the outside (**Figure 2B**).

Slide Panel

An opening in the panel (20 × 20 cm) was blocked by an opaque specifically-shaped hatch (ca. 28 cm wide on the top and bottom and 20 × 20 cm in the middle) that was inserted into a frame system on the top and bottom of the panel. To access the food, the hatch had to be slid sideways in the frame, either to the left or to the right (**Figure 2C**).

Step Panel

An opening in the panel (20 × 20 cm) was blocked by an opaque hatch of the same size that was attached with a hinge on the bottom and held in place by a magnet on the top back side. On the bottom side an elongated step pedal (10 × 5 cm) was attached to the hatch at a 90° angle. To access the food, the pedal had to be stepped on or pushed down to disengage the magnet, causing the hatch to fall open to the outside (**Figure 2D**).

In the test sessions, the food reward was put into the TBA, the two plain opaque panels were inserted into frames S1 and S3 and one of the four test panels was inserted into frame S2 so direct access to the food was blocked. In general, there was no consistent test protocol for this proof-of-concept study with regards to the order of the presented test panels and the exact length of each test session. We aimed to present each test panel 10 times to each group. The order was altered between both groups and decided *ad hoc* with the stipulation that the same panel was not used twice on the same day. Test sessions had a minimal duration of 30 min when the problem was not solved but sometimes ran longer (success after 30 min did not occur). For practical reasons, sessions were sometimes, but not always, stopped as soon as a hen was successful and the problem was solved. Whenever a successful test session was not stopped, the TBA merely became a food location for the remainder of the time. The side S2 holding the respective test panel and its immediate surrounding were filmed in each test session (Aiptek AHD H12 Extreme Camcorder).

TABLE 1 | Overview of success rates, mean solving times, and goal-directedness in each group.

	Test panel	Success rate [%]	Mean solving time [min:sec]	Goal-directedness [%]
Group 1	String	100	13:48	20
	Swing	80	10:17	75
	Slide	70	07:12	100
	Step	0	–	–
Group 2	String	100	01:38†	37.5†
	Swing	90	06:26	100
	Slide	60	21:12	100
	Step	10*	23:54	0

Note that solving times and goal-directedness only apply to successful sessions.
**Malfunction of hatch; † Missing video did not allow analysis of two sessions.*

Analysis

We analyzed 10 sessions per test panel per group, so that there were 40 test sessions per group in total. Analysis of the videos included success (yes/no), time of success (min:sec; starting point was always when the test panel was inserted and the experimenters left the TBA after baiting), and whether a successful individual proceeded to obtain the food reward within a few seconds after solving the problem ("goal-directedness": yes/no). The behavior and engagement with the TBA of the groups could not be analyzed in detail due to the limited camera angle but informal live observations were made. We tried tentatively to identify successful individuals of the groups from the videos. However, this was only possible in very rough terms, for example by clear differences of plumage color or conspicuous bald spots in the plumage.

To analyze successful problem-solving, we first compared the success rates on the four panels between the two groups using a Pearson's Chi-squared test in a 4×2 matrix (i.e., 4 panels × 2 groups). As no significant differences (*P*-value threshold 0.05) appeared across the success rates between the groups, we merged their data for the subsequent tests. Then, we tested whether the success rates across the four panels differed using a Pearson's Chi-squared test in a 4×2 matrix (i.e., 4 panels × success yes/no). For pairwise *post-hoc* comparisons we compared the success rates on the different panels with each other using Fisher's Exact Test for Count Data in 2×2 matrixes (i.e., 2 respective panels x success yes/no). All tests were calculated with R 4.0.3 (R Core Team, 2020; R code of the analyses is provided in the **Supplementary Material**). Behavioral observations were reported and the latencies and the "goal-directedness" of the successful birds were presented in a descriptive manner (see **Table 1**).

RESULTS

In both groups of laying hens, the animals habituated to the TBA with open frames immediately and learned quickly that it held a preferred food reward. A majority of hens was highly

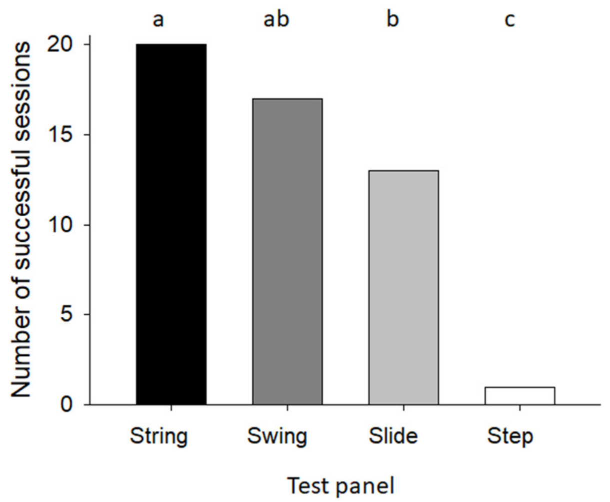

FIGURE 3 | Merged successful sessions of both laying hen groups for the four test panels: swing panel (black), string panel (dark gray), slide panel (light gray), and step panel (white). Different letters above the bars indicate significant differences in pairwise *post-hoc* comparisons between the test panels. The single success with the step panel was due to a malfunction of the hatch.

motivated to approach and engage with it to obtain the food. During baiting in test sessions, we observed that almost all hens were in close proximity to the TBA, limited only by the crowded space. Numerous birds explored and pecked the device with their number declining over the course of a session when no bird successfully solved the problem. Conservatively, there were at least five individuals in each group that solved at least one problem and there were single individuals that solved more than one problem. The problems were always solved by a single successful individual, not by a "group effort." However, considerably more than five individuals approached and engaged with the TBA in every session.

We found a similar gradient of success levels across the four test panels in both groups and the success rates between the two groups did not differ ($\chi^2 = 1.17$, df $= 3$, $p = 0.77$).

The string panel was solved in every session in each group but successful animals proceeded to obtain the food reward afterwards in only 20 and 37.5% of successful string panel sessions, respectively. The success rates with the swing panel and the slide panel lay between 60 and 90% and successful individuals had a high rate of immediate reward in these sessions (75–100% of instances). The step panel was solved only once in one group and this was due to a malfunction when the hatch opened after a light touch (see **Table 1** for more detailed results). The success rates for the four test panels differed significantly ($\chi^2 = 26.85$, df $= 3$, $p < 0.0001$; **Figure 3**). The pairwise(*post hoc*) comparisons revealed that success rates between string panel vs. slide panel ($p = 0.008$), string vs. step ($p < 0.0001$), swing vs. step ($p < 0.0001$), and swing vs. step ($p < 0.001$) were significantly different from each other, while string did not differ from swing ($p = 0.23$) and swing did not differ from slide ($p = 0.27$) (see **Figure 3**).

DISCUSSION

Our study aims have been met in the first application of the TBA device. We found that (1) three of the four test panels were solved at high success rates. At least 10 individuals had the agency and control to spontaneously solve at least one novel problem to gain a reward. We observed the majority of hens to be highly motivated to approach, explore, and engage with the TBA whenever it was baited. We also found (2) that the exchanging of panels and baiting proved to be quick and easy for the experimenters and hardly affected daily caretaking routines. As a proof of concept, it was a promising success and demands further exploration of this device as a cognitive enrichment and research method.

There was a significant gradient of success rates with the string panel being the easiest (100% success rate in both groups), followed by the swing panel (80 and 90%), the slide panel (60 and 70%), and finally the step panel that was never solved by means of the intended mechanism. While adjustments might be needed if the difficulty of the step panel turns out to be too high in general, a gradient of success rates is promising for more controlled studies as it can reveal variety and individual differences. The avoidance of ceiling and floor effects is a prerequisite for the design of validated cognitive test batteries (see e.g., Völter et al., 2018), for example with a "mini test battery" approach as a first building block (Schmelz et al., 2015; Shaw and Schmelz, 2017).

An interesting finding of the current study was the fact that successful animals with the string panel more often than not did not obtain a food reward immediately afterwards. In comparison, successful individuals with the swing panel and slide panel almost always proceeded to obtain the food reward. This is most likely due to the hatch of the string panel opening to the outside, so that bystander animals could enter the TBA faster than the ones opening it. However, this also (by chance) created an interesting variation across the different panels, as successful animals were mostly, but not always the ones being rewarded immediately. Because of this, monopolization was precluded and it suggests that the engagement with the TBA might potentially have been intrinsically rewarding without a direct food reward (Clark, 2017). Future studies should investigate if and to what extent animals keep on engaging and solving problems when there is consistently no food reward or when they can choose to obtain identical food without "working" for it (see Langbein et al., 2009).

As our approach (1) engaged the laying hens' cognitive skills by offering problems to solve and control over their environment while (2) providing extrinsic and potentially also intrinsic rewards, the TBA fulfilled at least two of the three conditions of Clark's (2011, 2017) definition of cognitive enrichment. With regards to the third—validated measures of wellbeing—we did not observe any aggression or injuries during test sessions. Any heightened arousal and spatial competition was arguably rather an indicator of positive eustress and challenge and therefore of successful enrichment (Špinka and Wemelsfelder, 2011). However, for the application of the TBA as a cognitive enrichment device in commercial farming, the validation and quantification of welfare benefits will be the most crucial aim of further studies. Aggressive group competition, monopolization by dominant animals, negative stress, and other adverse effects of its application must be excluded. One approach could be a comparison of behavioral and physiological measures between groups with and without access to this device. Additionally, the TBA is adjustable with regard to the size and the panels to be applied to other farm animals for a validation of its wider use as cognitive enrichment, for example to different species of poultry such as turkeys (*Meleagris gallopavo* domesticus) or mammalian species such as pigs (*Sus scrofa* domesticus).

In laying hens, our proof of concept must be expanded with a more standardized and controlled study protocol than the current study and the possibility to identify individuals, for example by wing tags that can be differentiated on video. We could not analyze in detail how many hens of each group engaged and were actually successful with the TBA in this initial study. Based on our informal observations a majority of the hens approached and engaged with it at least during baiting but a formal confirmation and quantification is crucial for an enrichment and research method as both are only effective when more than a small subset of animals is included.

Finally, further research must validate which cognitive or non-cognitive factors were targeted by our four problem-solving tasks and thereby add stronger insights to our knowledge of chicken cognition. This also contributes to the ongoing discussion of operant problem-solving studies and what they actually test (see e.g., Griffin and Guez, 2014; van Horik and Madden, 2016). While group-level testing has advantages (e.g., the familiar environment causing lower stress, ease of testing, and application in farm settings), individual tests of single animals separated from the group to engage with the TBA on their own will be needed. It allows a comparison of differences on an individual level to investigate if the hens engage in similar ways with the TBA and if individuals show consistent success rates across tasks and over time. With additional behavioral observations of non-cognitive factors like activity and exploration we can then correlate these to their problem-solving skills and success rates (see van Horik and Madden, 2016). Individual tests compared directly to group tests can also reveal how many individuals are successful with free access to the TBA and more time before a problem is already solved by another individual.

In conclusion, the laying hens in this study could have been unsuccessful or avoiding the TBA altogether. The practical application could have been complicated and time-consuming. However, this was not the case. Even though further research is needed, the TBA is a promising novel approach both for animal cognition research and for cognitive enrichment in farm animals in general and laying hens specifically. In our proof of concept, we could show that it is cheap to build and easy to apply with minimal disruption of daily routines in farming. By design, it is flexibly expandable and its potentially limitless configurations and modifications allow diverse opportunities for cognitive and behavioral research and enrichment that can be novel and challenging in the long term. The TBA is simple in its implementation but complex in its possibilities.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the study device was merely provided as enrichment and none of the animals was handled or restricted at any time for this study. Laying hens were kept in accordance to the German laws (TierSchNutzV) and housing and management were conducted to respective farming procedures with the permit no. DE276033510060555. Commercial layer feed and water were provided *ad libitum*.

AUTHOR CONTRIBUTIONS

MS designed the basic idea, apparatus, and procedure, collected and analyzed the data, and wrote the manuscript. EK contributed to the apparatus and procedure, collected and analyzed the data, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2021.671905/full#supplementary-material

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REVIEW
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Green Milk From Contented Cows: Is It Possible?

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The dairy industry is open to criticism on several fronts: obesity and ill health among the affluent, high demand for crops that could be consumed more sustainably and more equitably by ourselves, environmental damage and climate change, and abuse of animal welfare through production diseases and denial of normal patterns of behaviour. All these criticisms are valid. It is necessary therefore to examine in depth the nature and extent of specific problems to see which, if any, are inevitable, which can be mitigated and which can be avoided altogether. Dairy cattle, like all ruminants, can be sustained wholly, or in part on complementary feeds; grasses and crop residues that cannot be fed directly to humans. Fed appropriate diets dairy cows can produce more energy and protein for human consumption than they consume. The greenhouse gas, methane is an inevitable consequence of rumen fermentation. High yielding cows in confinement produce less methane per litre of milk. There is some scope for reducing methane production through manipulation of rumen fermentation but the impact is likely to be small. The most serious welfare abuses can be linked to genetic and management strategies designed to maximise milk yield from individual cows. These manifest in production diseases and metabolic exhaustion, both leading to premature culling. All these problems; too much milk, too much food waste, too much methane, too many stressed cows, are matters of degree. The poison is in the dose. Thus, solutions will not come from radical advances in biological science but public and political exercises in moderation.

Keywords: Livestock's Long Shadow, greenhouse gases, emergy analysis, genetic selection, welfare

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Give a man enough to eat, he has many problems, give him not enough to eat, he has only one problem.

- John Webster

MILK PRODUCTION IN THE CONTEXT OF THE GLOBAL DEMAND FOR FOOD

The essential needs of humans and all animals for the right amount of the right sort of food are immediate, continuous, and have long term consequences for the quality of our lives and that of the planet. Those with too little to eat are unable to promote normal development and good health for themselves and their offspring. Those who eat too much accelerate their rate of decay. The Food and Agriculture Organisation (FAO) (2006) states that 9% of the world population (820 million people) are experiencing severe hunger, two billion (22%) experience moderate to severe food insecurity [Food and Agriculture Organisation (FAO), 2020]. More than 20% of children under five show stunted growth. At the same time 13% are described as moderately to severely obese. Furthermore, things are getting worse at both ends of the scale.

Increasing numbers of humans self-evidently increases demand for food and puts increasing strain on the capacity of the living environment to meet this demand. However, population numbers *per se* present less of a strain on resources than increased demand from those with money to spend on attractive but environmentally spendthrift sources of food, especially that from animals given food that we could have eaten ourselves. It has been calculated that if the Chinese consumed the same amounts of the same sort of food as eaten by citizens of the USA (as is their right) they would exhaust the resources of the planet in <30 years. Radical change in the way we produce, distribute, and consume food, especially food from animals, is not just a moral aspiration; it is an ecological necessity.

It is self-evident that food from animals puts a greater demand on resources than food from plants, since so much of that food is required to maintain the animals themselves. This simple conclusion has laid the foundations for a number of complex analyses of resource use such as "Livestock's Long Shadow" [Food and Agriculture Organisation (FAO), 2006]. These describe the relative inefficiencies of using animals to exploit resources of sun, soil and water to produce food for human consumption, the degradation of these resources through overuse, the pollution of land and water from excessive waste products and the threat to the climate from greenhouse gases, especially methane from ruminants, which has ∼20 times the global warming potential of carbon dioxide.

Public criticism of the scale and practise of current methods of farming animals for food is based on the following four premises.

- Most of those who can, consume too much meat and milk.
- Food that we could eat is fed to animals while the poor go hungry.
- Livestock's long shadow is destroying the planet.
- Intensive livestock production is incompatible with animal welfare.

THE DAIRY INDUSTRY

This paper considers the dairy industry, which has become a major target for criticism on all four counts. Given the current state of the industry, much of this criticism is justified. However, it was not ever thus and there are sound reasons why it need not be so in the future. As a child at school, I got free milk, not always a treat on a summer's day after it had sat for some hours in the sun. This policy was a consequence of the classic work of Sir John Boyd Orr in the 1920s who demonstrated that much of the differences among children in both growth *and educational attainment* could be resolved by improved provision of essential nutrients provided by milk. For most of us today milk *per se* has become just a commodity, cheaper to buy than some brands of bottled water. The expansion of the dairy industry has been driven largely by the increase in demand for luxury foods, butter, cream, and a huge variety of ice creams, yoghourts, and cheeses. These luxuries are acceptable to lactovegetarians because they don't directly involve the killing of animals.

All this is very recent history. For most of recorded history, getting enough to eat was a struggle for survival and the cow was a highly valued partner in this struggle. The traditional role of the family cow was to provide milk, work, fertiliser, fuel, clothing, and the occasional fatted calf for special occasions, while sustained by fibrous feeds that the family could not digest for themselves, usually from land that the family did not own. She was not competing with the family for food; she was an essential contributor to the harvest. The modern dairy cow, typified by the Holstein breed, is a very different creature: bred, fed, and managed to produce as much milk as possible within intensive, highly mechanised dairy units. Meat production has become a relatively minor consideration, with male calves destined for beef or veal sent, more often than not, off farm to other specialist rearing units. Other traditional roles for the family cow have disappeared altogether. The modern Holstein is most unlikely to be harnessed to a plough! However, while the high yielding cow, confined in a barn, has become the norm for much of the urbanised, affluent human population, the proportion of cows kept in such intensive conditions in the less developed nations is relatively low.

Table 1 [Compassion in World Farming (CIWF), 2020] provides an illustration of the range of cow numbers and milk yields in different regions of the worlds. The highest yields are recorded in USA, Israel, and Saudi Arabia, in the latter two nations, especially, demand is high but the land and climate are entirely alien to the concept of milk from pasture. The EU embraces a range of production systems from the highly intensive in (e.g.) Denmark and the Netherlands to pastoral systems in Ireland and much of Eastern Europe. Here average annual milk yield is 5,900 l (range: Denmark −8,400 l, Bulgaria 1,850 l). New Zealand is included in **Table 1** as an example of an advanced industry producing milk primarily from grass. Average annual milk yield from the world population of 264 million dairy cows is only 2,270 l. The first message to be drawn from this table is that the dairy industry, unlike for example the broiler chicken industry, cannot be viewed, criticized, or applauded as a single homogenous system. The second message is that the dairy cow is a creature of infinite variety that can adapt to a wide range of husbandry methods. This creates the potential to adopt a constructive approach to all four of the concerns outlined above.

The four charges on which the dairy industry stands accused: unhealthy, unfair, unsustainable, and unkind, are not without substance. However, they are (obviously) simplistic because they do not begin to address all the variables in a complex industry. I shall not attempt to rebut these accusations but address them objectively and explore possible pathways to greener, kinder solutions.

AN UNHEALTHY DAIRY INDUSTRY? MOST OF THOSE WHO CAN, CONSUME TOO MUCH MEAT AND MILK

This has to be the biggest concern because it involves decisions faced by all humans as potential consumers, as against the shrinking minority who are directly concerned with the

TABLE 1 | Total milk production (million tonnes), cow numbers (millions), and average milk yields of cows (litres per cow per year).

	Total milk production (million tonnes)	Cow numbers (millions)	Average milk yields (litres/cow year)
USA	87.	9.1	9,600
European Union	135.5	23.0	5,900
India	50.3	43.6	1,150
New Zealand	17.0	4.6	3,700
World	599.4	264.4	2,270

production of food from animals. For the purposes of analysis, we need to distinguish three distinct concerns: the impact on our own health and welfare, on the health and welfare of the farmed animals and on that of the living environment. The first concern; that of human health and welfare, is largely outside the scope of this paper. However, it is important to view it not only through the eyes of those of us whose income and eating habits may be ranked as adequate to excessive, while approximately one quarter of the world population is chronically or intermittently short of food [Food and Agriculture Organisation (FAO), 2020]. A larger number can get enough to eat but are likely to experience malnutrition because their diet is dominated by starchy grains and roots. For these people, especially child-bearing mothers and their children, foods of animal origin, rich in protein, fats, minerals, and vitamins, are critically important (if not strictly essential) to health and well-being. Milk and eggs appeal to vegetarians because they do not involve killing the animal to get at the food. In a more biological context, milk and (unfertilised) eggs cannot be defined as bits of animals, but simply as foods of the highest nutritional value: the former to feed the growing calf from the time of its birth, the latter to feed the fertilised embryo up to the time of hatching.

The risks to human health of overindulgence in rich foods of animal origin have been researched and documented at great length but they are out with the scope of this paper. For practical purposes the sum of knowledge as to human nutrition may be condensed to eight syllables: "eat food, not too much, mostly plants" (Pollan, 2008).

AN UNFAIR DAIRY INDUSTRY? FOOD THAT WE COULD EAT IS FED TO ANIMALS WHILE THE POOR GO HUNGRY

This is more than an expression of concern; it is a fact: but it is not an inevitability. When considered in nutritional and ecological terms, the costs and benefits of food from animals are governed by the extent to which they may or may not compete with us for resources. The greatest of the essential demands of animals on resources is for energy from food to fuel the processes of life. This is best described in terms of Metabolizable Energy (ME MJ/kg dry matter) since this defines the amount of fuel that can be extracted from the diet by the processes of digestion and metabolism. In an adult animal in energy balance, neither gaining nor losing weight, all ME is used for maintenance and converted into heat. ME consumed in excess of maintenance is retained in the body as protein and fat or, in a lactating animal, secreted as milk. Food production from animals is inevitably less efficient than that from plants because much of the food eaten by animals is required to meet their own needs. At maintenance, the gross efficiency of conversion of animal feed into food for human consumption is zero. The *net* efficiency of conversion of increments of ME fed above maintenance may range from ∼0.6 to 0.8. The overall *gross* efficiency of conversion of energy in animal feed to energy in animal product (meat, milk, or eggs) increases with increasing ME intake to a limit set by physiological constrains on appetite. The limit to appetite in highly productive farm animals grown for meat is about three times maintenance and gross efficiency of conversion about 0.3. High yielding dairy cows may consume ME at 5 times maintenance and achieve a *gross* efficiency of 0.5. For further explanation see Webster (2016).

Table 2 compares the efficiency of conversion of feed energy (ME) and protein into hens' eggs, cows' milk, pork meat from the offspring of sows giving birth to 22 piglets/year and beef from extensively reared cow-calf systems where the contribution of the breeding beef cow is one calf/year plus her own carcass at eventual slaughter. In each column the efficiency of conversion of feed energy and protein is expressed in two ways:

- Overall efficiency: food energy and protein (for human consumption) relative to total ME and protein consumed by the productive and support animals. For meat animals these correspond to the slaughter and breeding generations. For the dairy industry they correspond to the lactating adults and replacement heifers.
- Competitive efficiency: food energy and protein (for human consumption) relative to animal consumption of ME and protein from "competitive" feed sources (i.e., feeds such as cereals that could have been fed directly to humans) as distinct from "complementary" feeds (grazing, forages, and by-products remaining after preparation of food and drink for human consumption (e.g., maize gluten, brewers' grains).

The *overall efficiencies* of ME conversion into eggs, pork, milk, and beef are 0.33, 0.19, 0.42, and 0.08, respectively; for protein conversion they are 0.32, 0.25, 0.28, and 0.09. The reason why the efficiency of energy conversion to milk is greater than that for egg production can be attributed to the fact that there has, to date, been no limit to the ability of breeders to select cows to produce more and more milk, whereas hens are still restricted to the production of one egg per day. Both milk and egg production are more efficient than the intensive production of pork meat: beef production (by these measures) fails to achieve an efficiency of 10%.

When energy conversion is examined in terms of *competitive efficiency* the picture changes. Here beef becomes as efficient as pork (or no less inefficient) and dairy farming becomes very efficient indeed. In this example, based on a typical diet fed to cows in the pasture-rich South West of England ∼65% ME is complementary and the output of food energy for human consumption is 39% greater than their demand for feed that we could eat ourselves. The ability of the dairy cow to produce

TABLE 2 | Efficiency of energy and protein conversion in meat, milk, and egg production (from Webster, 2013).

	Eggs	Pork	Milk	Beef
Production unit	1 hen	22 pigs	1 cow	1 calf
Support unit	0.05 hens	1 sow	0.33 heifers	1 cow
Output/year (kg food)	15	1,300	8,000	200
Food energy (MJ)	130	13,000	28,000	2,500
Protein (kg)	1.65	208	264	32
Input/year (MJ ME), total	389	67,038	67,089	29,850
"Competitive"	351	53,630	20,127	10,268
Input/year (kg protein), total	5.2	818	946	361
"Competitive"	5.0	736	236	108
Efficiency				
Food energy/total feed ME	0.33	0.19	0.42	0.08
Food energy/"competitive" feed ME	0.35	0.24	1.39	0.24
Food protein/total feed protein	0.32	0.25	0.28	0.09
Food protein/"competitive" feed protein	0.33	0.28	1.12	0.30

For each system, efficiency is described by the ratio of output to input, where output is defined by energy and protein in food for humans; inputs are described in terms of total and "competitive" intake of ME and protein, where "competitive" describes energy and protein from feed sources that could be fed directly to humans.

more food for human consumption than she eats is most marked in advanced pastoral systems as seen in New Zealand but can be achieved in fully housed systems though proper selection of complementary feeds.

Table 2 provides a powerful illustration of the fact that it is possible within modern, highly productive production systems to exploit the ages-old capacity of the milch cow to contribute, rather than compete in the constant endeavour to provide good food for ourselves, both rich and poor. It would be a mistake however to assume that because it can be done, it is being done. The present state of dairy production, especially in the rich, urbanised nations, involves far too much land to grow crops like energy-rich cereals and protein-rich beans and seeds to drive dairy cows to produce more milk than is compatible with health and welfare for them, us and the planet at large.

AN UNSUSTAINABLE DAIRY INDUSTRY? LIVESTOCK'S LONG SHADOW IS DESTROYING THE PLANET

This is a fiendishly complex issue to address because, by definition, it has to embrace all of life. Attempts to achieve a comprehensive assessment of the inputs, outputs, and environmental impact of any biological or industrial process are conventionally based on the principles of *life cycle analysis* (LCA). The International Organisation for Standardisation (IS0 14040) defines LCA as the study of "environmental aspects and potential impacts throughout a product's life cycle from raw material acquisition through production, use, and dismissal. Environmental impacts needing consideration include resource use, human health, and environmental consequences." This is easier said than done because, as defined, it includes everything, including much that we cannot measure with any certainty and much that is subjective. Any manageable approach to an LCA will posit specific questions and select data that would seem to be most relevant to these questions. When researching the literature on resource use and environmental impact of livestock production systems, it is unsurprising therefore to discover a wide range of conclusions and opinions among authors all using valid scientific methods. This can usually be attributed to the fact that they have posed broadly similar questions but in slightly different ways. My (similarly non-comprehensive) approach to LCA in livestock production systems will focus on two of the most important issues, energy use and carbon balance, especially the *net* production of carbon dioxide and other greenhouse gases (GHG) especially methane.

The Food and Agriculture Organisation Report "Livestock's Long Shadow" [Food and Agriculture Organisation (FAO), 2006] catalogues in great detail the ways and the extent to which livestock production, carried out in the manner and at the scale that exists today, is creating an unsustainable burden on the living environment. Ruminants are singled out for special criticism because of their contribution to global warming through the emission of greenhouse gases (GHG), especially methane produced from fermentation of fibrous feeds in the anaerobic environment of the rumen, Methane has ∼25 times the global warming potential of CO_2. The LLS report considers ways to mitigate this and other environmental threats from land degradation from overgrazing, and pollution of land and water from nitrogenous wastes within current intensive systems operations at current levels of production. A grossly oversimplified take home message from their conclusions would be that it is best for the environment to eat eggs, poultry, and pork reared intensively indoors. In my opinion however, the LLS report falls short on several counts. It dodges the central issue, namely that our current problems arise not from livestock production *per se*, which has been an integral part of sustainable livestock production for millennia, but the current scale of livestock production, both intensive and extensive, that grossly disrupts the ecological balance. To give an obvious example: nitrogen pollution from agricultural waste is simply a case of too much fertiliser in the wrong place. LLS calculates the environmental cost of production systems in terms of global hectares of land required to produce a standard amount of different foods for humans of plant and animal origin but does not adequately take into account the differing capacity of different classes of land to produce crops, e.g., grasses vs. cereals. It does not properly account for such things as differences in the availability and therefore the value of site-specific resources, most especially, water. Problems of water supply and disposal are very different for dairy units in Israel and the west of Scotland. It does not fully consider the extent to which the effects of the emission of GHG may be offset by carbon sequestration in pasture and woodlands grazed and browsed by ruminants.

My main objection to the LLS report is that while it considers strategies for mitigating environmental costs within the context of current production methods and consumption levels, it gives little attention to the extent to which livestock husbandry, using appropriate species in sustainable numbers can, at best, make a positive contribution to environmental quality or, at least, greatly mitigate the costs.

LIFE CYCLE ANALYSIS: ENERGY AND CARBON INPUTS, OUTPUTS, AND EMISSIONS

Tara Garnett and her colleagues have produced an excellent overview, "Grazed and Confused" (Garnett et al., 2017) of the impact of ruminant production systems on the climate and living environment. This considers all the costs, such as GHG emissions and the nitrogen pollution of waterways but it also gives proper attention to ways in which pastoral systems, properly managed, can enhance the quality of the land. In this section, I apply the principles of partial life cycle analysis (LCA) in an attempt to quantify and compare carbon and energy exchanges in livestock production systems. The main carbon and energy inputs are feed and fuel, the main products are food (milk and meat) and "wastes" principally nitrogenous wastes in manure and greenhouse gases (GHG) released into the atmosphere.

A substantial weight of literature has accumulated in respect to net GHG emissions from livestock production systems. Net GHG emissions describe the algebraic sum of GHG production as CO_2 and CH_4 (mostly from animals and manure) set against carbon sequestration in land grazed by the cattle. Plants convert atmospheric CO_2 into organic matter by the process of photosynthesis. Carbon is stored in the plant, above and below the ground, so long as the organic matter continues to exist, alive, or dead. Selectively felling forest trees to build houses or battleships stores C and gives other trees space to grow and store more. Slashing and burning the jungle to clear space for soya or palm oil production brings double jeopardy: it releases all the C into the atmosphere and radically reduces the future capacity for C storage. Untouched tropical rain forests, where nearly all C is retained within the system as organic matter sequester C long-term. A high proportion of C captured by photosynthesis is stored as organic matter within the soil. It follows that soil erosion is a major contributor to GHG production. The soil under permanent pastures of mixed grasses and clovers will store much more soil organic carbon (SOC) than arable land used for intensive production of cereals and oilseeds. However, there is a limit to the amount of C that can be stored so that, in time, an equilibrium is reached where net C exchange between plants and atmosphere is zero.

Most of the carbon-based fuels upon which we depend today were laid down during the carboniferous era. At the beginning of this period atmospheric CO_2 concentration was about 20 times the concentration of 3 parts per million (ppm) recorded at the beginning of the Anthropocene in about 1850, when human mining and consumption of fossil fuels began its it long ascent. The climate at the start of the carboniferous period was hot, wet, and most of the planet was under water. Most of the land consisted of tropical rain forest, which sequestered nearly all the carbon it captured. By the end of the carboniferous period of 60 million years atmospheric CO_2 had fallen below 2 ppm. Atmospheric oxygen (currently 21%) was over 30%. Physics dictates that this period of climate instability must have ended in a catastrophe and it did: an ice age.

Table 3 presents a condensed and greatly simplified summary of data gathered by Pelletier (2008) and Pelletier et al. (2010a,b) to illustrate the application of LCA to calculate energy use and production of GHG in meat production systems in the USA. The examples include three intensive (commercial) systems, broiler chickens, pork, and feedlot beef and two more "natural" systems; "niche" pork (equivalent to organic) and beef cattle finished at pasture. The numbers are expressed to only two significant figures, given the high dependence on assumptions, even this approximation almost certainly implies a greater degree of accuracy than is warranted in terms of the absolute numbers. However, the same rules and assumption are applied throughout so the comparisons between systems may be treated with confidence. While the less intensive systems may have relied to a greater extent on complementary feed sources, fuel energy costs were significantly greater. GHG production was conspicuously greater from beef cattle finished at pasture than in feedlots. Similar conclusions may be drawn from a recent paper by Pieper et al. (2020) that estimated organic production methods for poultry and pork in Europe generate ∼50% more GHG than intensive methods.

These comparisons should be treated with caution since they are specific to the production methods that they describe and cannot be applied worldwide. The high fuel costs for pasture-finished beef cattle in the USA reflect the high of nitrogenous fertilisers. Nevertheless, they illustrate the important point that more "natural" methods are likely to be less sustainable *according to these criteria* mainly because slower growing animals have a lower gross efficiency of utilisation of ME (as described above) and produce more GHG equivalents per tonne of meat for human consumption. However, this analysis, like all partial LCAs is based on limited, selected premises. It does not, for example, take into account the impact of organic farming methods on soil quality, ecological diversity including sentient wildlife or, of course, the welfare of the farmed animals.

There is a weighty volume of literature on GHG emissions from dairy cattle. Methane production is a consequence of anaerobic fermentation in the rumen, thus most of the effects of productivity and nutrition can be derived from first principles. Increasing individual milk yield decreases the amount of GHG produced per litre milk as the proportion of digestible energy directed to milk production increases with respect to that required for maintenance (see **Table 2**). Higher yielding dairy cows are fed a diet containing a higher proportion of starch to cellulose in the diet and this increases fermentation to propionate relative to acetate. This reduces the proportion of fermentable energy that is lost to the system in the form of "excess" protons converted to methane. These basic principles are explained in greater depth in "Understanding the Dairy Cow" (Webster, 2020). Estimates of the extent to which diet and production level can reduce GHG emissions relative to milk yield include Gerber et al. (2010). Conceptually, the most promising approach to reduction of GHG is through manipulation of the ruminal

TABLE 3 | Life cycle analysis of energy inputs and emissions of greenhouse gases (GHG, measured as CO_2 equivalents) in the production of 1 kg of meat in broiler chicken, pork, and beef production systems (after Pelletier, 2008; Pelletier et al., 2010a,b).

Output (1 kg meat)	Energy use (MJ)			GHG (kg CO_2 equivalents)
	Feed	Fuel	Total	
Broiler chicken	10	5.0	15	1.3
Pork, commercial	6.1	4.9	11	2.7
Pork, niche	7.1	5.9	13	3.2
Beef, feedlot finished	28	10	38	35
Beef, pasture finished	41	7	48	46

microbiome through diet, pharmaceuticals or possibly genetic selection. A large international study of European dairy cattle has revealed heritable differences in the rumen microbiome that should affect methane production (Wallace et al., 2019) although it has not, as yet, presented direct evidence as to the degree to which this might be achieved in practise. A very recent paper by Roque et al. (2021) has demonstrated a sustained reduction of over 60% in methane production, with a concomitant increase in hydrogen production from beef cattle as a result of feeding red seaweed *Asparagopsis taxiformis*, which contains significant amounts of the trihalomethane, bromoform, a known inhibitor of methane production. If it can be confirmed as both effective and safe, this holds promise.

GHG emissions associated with milk production may be compared with those associated with an equivalent production of food from simple-stomached animals. Rotz et al. (2010) using data from dairy herds in California and Pennsylvania with annual yields ranging from 5,500 to 11,000 kg/lactation calculated values for GHG production of 0.4–0.7 kg CO_2 equivalents per litre of milk produced. To compare these values with those in **Table 3**, the energy value of 1 kg of meat may be taken as approximately three times that of 1 l of milk, so that GHG emissions from milk production correspond to 1.5–1.8 kg CO_2 equivalents per kg meat equivalent. By this measure, the GHG impact of milk production is intermediate between that of chicken and pork. Beef production, by any means, is extremely profligate.

Few, if any, of the soils in land currently used for agriculture are likely to be in a state of C equilibrium. Evidence based on measurements of soil organic C show that while much of the arable land used for the intensive production of cereals and oilseeds is losing carbon, European grasslands (for example) are currently sequestering C, thus acting as a sustained C sink (Soussanna et al., 2010). These estimates of net C balance (CO_2 equivalents per m^2 land) in European pastoral systems for dairy and beef production predict that the rate of C sequestration relative to GHG production increases with the proportion of feed that is directly grazed, so that *by this measure* extensive beef production from pasture-fed cattle becomes the most sustainable.

EMERGY ANALYSIS

Because all forms of life cycle analysis are partial they will inevitably lead to different conclusions according to the questions asked and the variables included in the model. To my knowledge, the closest approach to a comprehensive LCA of exchanges of energy and matter in any production system is that known as "*emergy analysis*," where emergy (E_m) is a measure of the amount of the original, effectively inexhaustible source of solar energy embedded at each stage of the process This concept expresses all the work processes and resources (sunlight, water, fossils fuels, minerals etc.) used in the generation of a product in terms of a common unit of measurement (Zhao and Li, 2005). The approach is fiendishly complex, and like most LCAs carries a lot of uncertain assumptions that but it is, I believe, particularly well-suited to the assessment of the efficiency and sustainability of farming the land for food because it can identify, distinguish and quantify the renewable (R) resources of sun, soil and water embedded in farmland from non-renewable sources (NR) such as fuel, fertiliser, labour, and imported feeds (**Figure 1**).

In the context of food production, resources are defined as follows:

- Renewable Emergy (R) = emergy equivalents from sustainable sources, e.g., sunlight, free water
- Unrenewable Emergy (UR) = loss of energy from (e.g.) soil degradation
- Purchased goods and services (F) = bought in feed, fuel, labour, etc.
- Yield (Y) food for human consumption

Table 4 compares yields and sustainability in different agricultural systems on the basis of the following ratios.

- EYR (emergy yield ratio) = (R + NR + F)/F. This describes the contribution of local resources (land) to product,
- ELR (environmental load ratio) = (NR + F)/F. This describes the ratio of non-renewables to renewables in product.
- ESI (emergy sustainable index) = EYR/ELR. This becomes a measure of yield relative to environmental compatibility)

Values for EYR show that the relative contribution of local renewable resources did not differ greatly between corn production, conventional and organic pig production. The contribution of local resources was greater for dairy production, especially low-intensity dairy production in South Mali. This is consistent with the evidence presented in **Table 2**. The grazing of beef cattle was by far the most efficient in terms of the contribution of renewable resources. The most striking differences between the systems are revealed in column 3, ESI, the measure of yield in relation to environmental compatibility. By this measure, small scale dairy production is more sustainable than intensive production even in Brittany where a large proportion of feed comes from pasture, and extensive beef production on the Argentinian pampas outstrips all others in terms of sustainability. This may come as a surprise to urbanised critics of livestock production and beef production in general, but it would appear as an overcomplicated proof of the obvious to the gauchos of the pampas or the indigenous races of North America living in perfect symbiosis with the bison.

The LLS approach to calculating the environmental costs of agricultural systems has been based primarily on land use

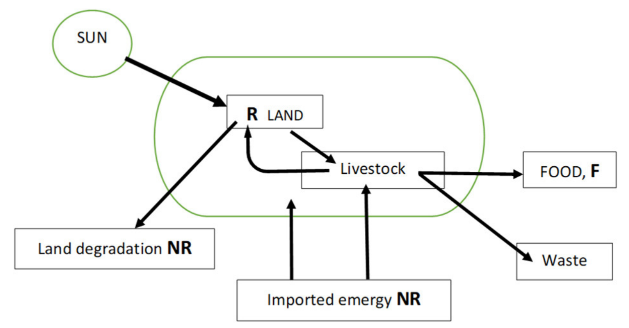

FIGURE 1 | The "Emergy" approach to the evaluation of renewable (R) and non-renewable (NR) sources of energy and matter to the production of food for human consumption.

TABLE 4 | Yield and sustainability within agricultural systems assessed in terms of embedded energy ("emergy") and described by three ratios (EYR, emergy yield ratio; ELR, environmental load ratio; ESI, emergy sustainable index).

	EYR	ELR	ESI
Corn (USA)[a]	1.07	18.8	0.06
Conventional pig (Sweden)[a]	1.04	22.3	0.05
Organic pig (Sweden)[a]	1.13	7.80	0.15
Intensive dairy (Brittany)[c]	1.35	3.25	0.42
Extensive dairy (Mali)[c]	1.89	1.25	1.57
Grazing cattle (Argentina)[b]	3.73	0.55	6.80

These ratios are dimensionless. Data taken from Pereira and Ortega (2013)[a], Rotolo et al. (2007)[b], and Vigne et al. (2013)[c]. For further explanation see text.

and concluded that the environmental cost of feeding people on beef is 10 times the cost of cereals and 40 times the cost of soya. The emergy approach yields a diametrically opposite conclusion (**Table 4**). By this analysis and in this example, corn and soya are the least sustainable because of their dependence on non-renewable resources of (e.g.) fertiliser and fuel (F) and degradation of soils (UR); beef from cattle grazing the pampas of Argentina are the most sustainable, both in terms of food emergy yield relative to the consumption of non-renewable resources (F/NR) and in terms of overall sustainability, defined by the Emergy Sustainable Index. I concede that the examples illustrated in **Table 4** have been chosen by me to make a point. Different approaches tell different stories. However, they all point to the same two conclusions. The first is that the current demand for foods of animal origin, particularly when this involves the feeding of animals of food that we could have eaten ourselves, is unsustainable. The second conclusion is that the key to sustainable farming is to manage different land types in ways that best respect the value of the location and land as defined by its own special resources of sun, soil, and water. This, indeed, is the essence of husbandry. Nobody, I hope, would consider ploughing up the Argentine pampas; nobody, at least, who is aware of the disastrous consequences of ploughing up the North American prairies that led to the dustbowl of the "dirty thirties."

It makes good ecological sense to derive value from land best suited to pastures through the production of food of high nutritional value from animals dependent, so far as possible, on complementary feeds that we cannot eat ourselves. It makes even more ecological sense in silvopastoral systems (Chará et al., 2019) where food production is just one of several contributors to value; others being income from sustainable forestry, water management, habitat and wildlife conservation and, not least, greater carbon sequestration. These forms of good husbandry cannot, however, produce meat and milk in the quantities that the comfortable and affluent have come to expect.

AN UNKIND DAIRY INDUSTRY? INTENSIVE DAIRY PRODUCTION IS INCOMPATIBLE WITH ANIMAL WELFARE

In recent years, the most common expressions of public concern as to methods of food production have related to issues of farm animal welfare. A particular target for criticism has been the industrialisation and intensification of animal production ("factory farming") that would appear to treat the animals as commodities rather than sentient beings. This concern was given expression by Ruth Harrison in her book "Animal Machines" (Harrison, 1964), a passionate and well-researched attack on the factory farming of broiler chickens, battery hens and veal calves reared in intense confinement. This led to the Brambell Committee of Enquiry into the Welfare of Animals kept under intensive housing conditions (Brambell, 1965). They recommended that all farm animals should be given sufficient space so that they are able, without difficulty, to "stand up, lie down, turn around, groom themselves, and stretch their limbs." This recommendation became known as the Five Freedoms. A combination of public pressure, good science, and legislation has stimulated the development of improved production methods within the highly intensive systems identified by Harrison and Brambell. Egg production in the UK is now based largely on "free range" systems to the satisfaction of the general public, if not necessarily the birds. Today, one of the most serious expression of public concern is that at a time when we have freed hens from the battery cage and given them free range, the dairy industry has taken the cows out of the fields and confined them on concrete. This is an oversimplified image, but it is a powerful one that needs to be addressed.

The publication of the Brambell report led to the formation of the UK Farm Animal Welfare Advisory Committee, subsequently the Farm Animal Welfare Council (FAWC). When I was first appointed to this committee in 1979, I argued that while the five freedoms as described by Brambell were of great importance, especially in the context of the extreme confinement systems considered within their brief, there was so much more to animal welfare then just space allowance. Following much discussion, FAWC produced a concise but comprehensive summary of farm animal needs and provisions (Farm Animal Welfare Council, 2006). These are:

- Freedom from hunger and thirst—achieved by readily accessible fresh water and a diet to maintain full health and vigour.
- Freedom from discomfort—achieved by appropriate shelter with a dry, restful lying area, and temperature within an acceptable range of tolerance.
- Freedom from pain, injury, and disease—achieved by prevention or rapid diagnosis and treatment.
- Freedom from fear—achieved by conditioning animals to their surroundings and avoiding situations that cause stress.
- Freedom to express normal patterns of behaviour—achieved by the provision of room to move, things to do and the company of their own kind.

These recommendations have stood the test of time. They are measures of *outcome,* now recognised as the most direct approach to the assessment of animal welfare. They are, moreover, not intended as a counsel of perfection but as a guide to good husbandry: simple enough to be memorable but comprehensive enough to be effective. Four of these five freedoms are freedoms *from,* and these have met with general approval. The final freedom: "to express normal patterns of behaviour" has aroused some concern. What, for example, is normal behaviour and when does normal behaviour become unacceptable? Isaac Stern expressed this well in a human context by pointing out that your freedom to swing your fist stops at the point of my nose. If I could persuade the successors to FAWC to rewrite the fifth freedom, I would re-express it simply as "freedom of choice." In practise, this implies (e.g.) freedom to select a preferred environment for rest and recreation, freedom to select and avoid contact with specific individuals in their social environment.

It is necessary to make the distinction between animal welfare and well-being. Welfare describes the physical and mental state of an animal across the whole spectrum from very good to very bad. Well-being describes a state within the range of satisfactory to good and must therefore be the aim of good husbandry. When measured strictly in terms of (short term) economics, large industrial dairy units have been an undoubted success. When measured in terms of the well-being of the animals and the land, achieved through sympathetic and sustainable husbandry, they are found to be wanting. The needs that drive the mind of the modern, highly bred, intensively fed cow are much the same as for any sentient mammal: food and water, comfort, security, and a stable social life consistent with the genetic imperative for sex. Fundamental to all these specific needs is freedom of choice: to take action to avert discomfort or threat and promote a positive sense of well-being. As we know too well, the impact of food on our state of mind is not just a matter of acquiring sufficient nutrients. So too with cows. The acts of eating and, in their case, ruminating, bring their own satisfaction. Grazing animals in the wild state have adapted to seasonal changes in food availability: lots of good grass in the summer or rainy season, much less food of much poorer quality in the winter or dry season. It is entirely natural for grazing animals to lose weight during the lean months, but provided some grazing is available, however poor the quality, they get the satisfaction of freedom to forage for what they can.

The most severe welfare problems for the dairy cow are likely to be associated with physical stresses to her physical and mental health rather than denial of behavioural expression. Relative to most farm animals she is most unlikely to suffer in consequence of having nothing to do all day. On the contrary she is worked quite extraordinarily hard. The modern dairy cow can cope in the short term with the intense metabolic demands involved in the production of 60 l milk/day (or more), coupled with the demands of consuming and digesting enough food to meet these demands. It is an inescapable fact, however, that too many succumb too soon to the long-term stresses of lactation, in particular, the *production diseases* such as rumen acidosis, ketosis and environmental mastitis and lameness that are, by definition, linked to the methods employed in the breeding, feeding, and housing of cows to produce large quantities of milk and therefore, by definition, our fault.

Table 5 presents a brief summary of potential welfare abuses that may occur in dairy systems. For the most part it is based on the template laid down by the five freedoms, but includes a further stress, namely that of *exhaustion* arising from failure to cope, in the long term, with the exacting physical demands of lactation. For the dairy cow, exhaustion is probably the biggest problem of all. It describes a cow broken down in body, and probably in spirit, through a combination of stresses associated with nutrition, housing, hygiene, and management exacerbated in many cases by breeding programmes that have overemphasised productivity at the expense of robust good health. Too many infertile, emaciated, or chronically lame cows are culled prematurely because they are no longer making a productive contribution to the enterprise. This is not only an abuse of welfare but also a terrible waste since a dairy cow needs to complete at least four lactations to recoup the cost of rearing her as a heifer until she delivers her first calf and enters the milking herd (Webster, 2020).

The most common breed of dairy cow in intensive systems is the Holstein. During the period 2002–2014, average lactation yields in UK Holsteins increased by 21% from 7,637 to 9,239 kg//head (AHDB, 2018). Within "elite" dairy herds in the USA average lactation yields in excess of 11,000 kg are commonplace. These increases have been achieved through a combination of selection strategies heavily weighted toward increased production of milk solids and developments in nutrition designed to support the high metabolic demands of lactation within the constraints of appetite. In simple terms, this involves increasing the ratio of cereals, where the main energy source is starch, and protein-rich oilseeds (e.g., soya, rapeseed) to forages (fresh and conserved grasses) where the main energy source is digestible fibre. Whatever their genetic potential, it is only possible to achieve these high yields if the cows are confined and forage intake is restricted. This policy inevitably presents threats to health and welfare.

Cows are not motivated to eat by a desire to reward the farmer with as much milk as possible, but by the desire to attain a feeling of comfortable satiety. Their capacity to take in food, especially fibrous food essential for healthy digestion, is constrained by the rate at which this food can be fermented in the rumen. Selection for increased yield increases the probability that they

TABLE 5 | Abuses of the five freedoms that can arise through systematic failures in the provision of good husbandry.

Hunger	Nutrition fails to meet the metabolic demands of lactation
Chronic discomfort	Ruminal indigestion
	Poorly designed cubicles, inadequate bedding
Pain and injury	Claw disorders (sole ulcer, white line disease)
	Digital dermatitis
	Damaged knees and hocks
Disease	Mastitis, ketosis,
Fear and stress	Rough handling, bullying, separation from calf
Lack of choice	Zero grazing, inadequate rest time
Exhaustion	Emaciation, infertility

will be unable to meet their metabolic demand for nutrients to sustain lactation and body condition within the limit of appetite set by the capacity and rate of digestion within the rumen. If they cannot eat enough to meet their metabolic demands, they will experience a sense of chronic metabolic hunger. To increase nutrient intake within the constraints of gut fill it is necessary to increase fermentation rate within the rumen by increasing the proportion of rapidly digestible starch to slowly digestible fibre. This increases the risk of ruminal acidosis, which is, at least uncomfortable and, in severe cases can lead to severe malaise and even death (Vigne et al., 2013). Many high yielding cows can simultaneously suffer from chronic hunger and the discomfort of ruminal indigestion. This is not a good feeling.

Cows' need for comfort is greatly influenced by their size and shape. The modern Holstein weighs over 700 kg and has prominent joints, especially knees and hocks. For comfort they need to lie down on pasture or a deformable bed of straw or sand. Concrete does not feel good. Cows are motivated to lie down to rest for about 11 hours per day (Norring and Valros, 2016). There comes a point where the need to lie down overrides the need to eat. In many intensive units high yielding dairy cows are milked three times daily, having queued to enter the milking parlour. They are also compelled to eat for at least 8 h to meet their nutrient demands. With so much to do, the time to lie at rest will be much less that they would wish.

Cows, like all sentient animals, are motivated by curiosity and caution. Curiosity is a powerful motivator in early life as calves seek to gather useful information. In later life, in a stable environment, caution becomes the wiser approach to ensuring a sense of security. Most cows in stable groups establish a stable hierarchy, through the exchange of social signals that usually avoid physical conflict. In houses where each cow has access to an individual cubicle it is normal for each to use the same cubicle every time. Overworked by the demands of lactation, they opt for the quiet life. However, they do retain their curiosity. If you wish to be entirely surrounded by curious cows, lie down in a field and the rest will follow. Horizontal, we present no threat and become interesting.

Whether on the family farm or in large intensive units, the dairy cow is a valuable individual and will be given individual attention. Despite this, dairy cows are at high risk of three major health problems, infertility, mastitis, and lameness. These conditions are known as production diseases, a phrase that concedes that they are largely our fault. Pryce et al. (1997) explored the genotypic and phenotypic links between selection for increase milk yield and the incidence of these three conditions. At that time there was a significant genotypic correlation between milk yield and all three. In the case of infertility and mastitis, there was no significant phenotypic link, which indicates that farmers were able to offset genotypic deterioration in these traits through improvements in management. In the case of lameness both genetic and phenotypic correlations were significant, which suggests that farmers were failing to hold the line. In recognition of the genetic link between selection policies heavily weighted toward increased yield, breeding companies have reformulated their selection indices to give increased emphasis to traits defined as robust as measured by an increase in productive life span (De Mello et al., 2014). In the selection index currently used by the UK Independent Dairy Breeding Company nearly 70% of traits are now based on measures of fitness, longevity and good welfare. The impact of selection for this set of traits on the progeny of tested bulls is integrated in the form of the *Profitable Lifetime Index.*[1] However, individual farmers can select bulls to suit individual cows and their individual systems by giving individual attention to specific traits related to resistance to the main production diseases: fertility, body condition, locomotion, and somatic cell counts (SCC), for resistance to mastitis.

There is, at present, no evidence to suggest that the incidence of production diseases is greater in large intensive units than on the traditional family farm. The incidence of infertility is linked to poor body condition, itself a consequence imbalance between the metabolic needs of lactation and the capacity of the cow to ingest and digest feed. While digestive disorders, especially rumen acidosis, are a major threat to the welfare of dairy cows, improvements in understanding of ruminant nutrition and the application of this new knowledge to the formulation of total mixed rations have done much to reduce the risks attached to the selection and management of high genetic merit cows to produce prodigious quantities of milk. The risk of physical discomfort, pain and injury in dairy cows attributable to poor housing and inadequate control of lameness is high. However, once again, there is no convincing evidence to suggest that these problems are worse in large, intensive units where cows are confined throughout lactation than in small family farms, where cows are at pasture during the summer. Indeed, the physical environment within large, new, expensive dairy units can often present a lower risk of injury than on the traditional, old, undercapitalised family farm.

There are some practises that we inflict on cows entirely for our benefit, in full knowledge that they conflict with how they would naturally perform to promote a sense of well-being. The top three, in ascending order of importance, are:

- Tethering cows throughout the time they are housed

[1]Profitable Lifetime Index. ahdb.org.uk>profitable-lifetime-index>pli.

- Keeping cows permanently housed, without access to pasture
- Removing calves from their mothers shortly after birth.

In many small rural communities it has been traditional to keep dairy cows outdoors all summer on lush pastures, like Alpine meadows, then bring them in for the winter and tether them in tie-barns where they will be fed, watered, and milked until turn-out in the spring. This practise has given rise to concern mainly on the grounds that it denies freedom of movement and opportunities for a social life. I know of no evidence that cows display signs of distress associated with prolonged tethering, although passing the winter group-housed in a barn with deep clean straw and access to an outside yard would undoubtedly be better. Some free-stall houses with insufficient, poorly bedded cubicles, and filthy passageways can be worse than tie-stalls. In any event, tie-stalls are incompatible with modern milking systems and will, I predict, gradually fade away.

In a few areas, such as UK, Ireland, and New Zealand people are accustomed to seeing dairy cows outdoors at grass during the summer, so assume this to be the natural state. However, this is becoming the exception through most of the developed world where the majority of lactating dairy cows are kept off pasture throughout their working life. The trend in commercial dairy production, world-wide, is toward industrialised units of 1,000 or more very high-yielding cows. In order to sustain these high yields, the cows are housed throughout lactation and given continuous access to a ration that ensures they take in far more nutrients than they could possibly derive from grazing at pasture because nutrient density of the ration is higher and the feed can be consumed more rapidly. Confinement also keeps the cows close enough to the milking parlour to permit thrice-daily milking or, increasingly, the use of a robot milking machine that they can enter of their own free will. This offers freedom of choice. However, robot milking machines are only practicable when cows are permanently housed. Mature cows do not appear to be strongly motivated to enter the milking parlour simply to relieve discomfort to their distended udders (Prescott et al., 1998). They need a food stimulus to attract them in from pasture. The attractions of pasture can be greater than the attractions of the robot milker, even when feed is on offer in the parlour. In consequence they visit the robot less often and milk yield falls. This makes it progressively easier for the cow to meet her metabolic needs from pasture so increases her preference to stay outdoors. Her welfare will improve but her productivity will fall. In some large, intensive units cows are confined throughout lactation but given a period of recreation on pasture for a few weeks during the dry period when they have completed their lactation and await their next calf.

Pasture provides an excellent source of nutrients in the form of fresh and conserved grasses and clovers. Moreover, when the weather is fine, pasture is an ideal environment for dairy cows. Here they can do much as they please: take in food, excrete urine and faeces, exercise, rest, enjoy fresh air and space, socialise and satisfy their curiosity. There is however a conflict between the use of pasture as a recreation area and the need to maximise its potential as a source of high-quality feed. Once the first flush of spring grass is over, most of the best grasslands are harvested for silage. For much of the so-called grazing season cows may be turned out onto "sacrifice" pastures that provide little nutrition but all the other amenities. In these circumstances pasture is serving only as a recreation area. The most cow-friendly farm I have ever seen was in the forested foothills of the Pyrenees in northern Spain. Cows could choose to roam in comfort and security among the trees, or rest in well-bedded kennels. There was little of nutritional value in the forest but much of interest. Nutrition, including freshly cut grass in season was provided at a feeding station close to the milking parlour. This "zero-grazing" system came as close as possible to meeting all their day-to-day needs, but it was exceptional. Ideally, cows should have freedom of choice to go outdoors, when they wish, where there is space, cool fresh air, and a comfortable place to rest. Cows are, undoubtedly highly motivated to graze fresh grass and I am always moved to watch the scenes of excitement when they are first turned out in spring but I cannot find strong evidence to indicate that they suffer from the inability to graze *per se.*

Our most extreme disturbance to the emotional state and natural behaviour of dairy cows is the policy of removing their calves shortly after birth, partly for ease of management but mainly to maximise income from sale of milk. It is difficult to estimate the possible magnitude of this practise in scientific terms. Comprehensive reviews of the literature relating to the effects of early separation on the behaviour (Meagher et al., 2019) and health (Beaver et al., 2019) of cows and calves reveal little of significance. We have little option but to consider the practise within the context of the natural behaviour of cow and calf.

Whether in the wild, or out-of-doors on the farm, the natural behaviour of the dairy cow at parturition is to separate from the herd and give birth in what she thinks will be a safe spot, for example, close to a hedge. Having licked the calf into shape and given it a first meal, she leaves it and returns to the herd; instructing it, in effect, to lie still and unnoticed until she returns to give it another drink. This behaviour is hard wired and has survival value. After a few days, when the calf has become active and can move as well as its mother, it will join the herd, spending much of the time with other calves, because they are more interesting, visiting its mother perhaps 4–6 times daily for a feed and usually resting with her at night. It is natural for cows and their calves to spend a long time apart, but both show signs of distress if not together at mealtimes. A few farmers separate cow and calf but allow the calf to join its mother twice daily to take a modest feed before the rest of the milk goes into the machine. This system appears to be acceptable to both mother and calf. Many domesticated water buffalo, e.g., in India, will not permit themselves to be milked unless their calf is present.

While I believe that the twice-daily access system is a reasonable approach to sympathetic husbandry, it is likely to remain a minority pursuit. What then is the least-worst approach to early weaning? In this context, the French word *sevrage* is more accurate. At present, the most common practise is to separate the calf within 24 h of birth. On some traditional dairy farms, calves will be left with their mothers for 2–3 weeks to ensure they get the full benefits of mother's milk. However, weaning after 3 weeks undoubtedly causes more distress to both cow and calf than weaning shortly after birth. Early weaning is an unpleasant business but, in the words of the murderous Macbeth "when 'tis done, it were well it were done quickly."

THE POISON IS IN THE DOSE

I have, so far, sought to address major sources of criticism of modern dairy practise arising from both the concerned general public and those with professional knowledge of the industry. In each case, I start from the premise that there is a case to answer then proceed to examination of the evidence. Some is taken from new science, and here I have included a small number of citations, as an introduction to further reading. However, most of my argument has been based on established scientific principles of nutrition, physiology, genetics, and behaviour, together with equally well-established practical principles of good husbandry. Selected references to original scientific communications would, I believe, add little to this element of my argument. Many points are explored in greater detail in the latest edition of "Understanding the Dairy Cow" (Webster, 2020).

The common theme that emerges from examination of this critique of the dairy industry is that problems are almost entirely problems of scale: "the poison is in the dose." Most of us who can, consume too much meat and milk for our own health and for the health of the planet. A significant reduction in our consumption of food from animals, especially those that are largely dependent on food that we could eat ourselves (e.g., cereals and proteinaceous beans and seeds), would greatly reduce the amount of land needed for growing crops and thereby improve the long-term quality and sustainability of the land through reforestation, rewilding and carbon sequestration, especially within the soil. A diet and lifestyle that excludes all food and other products of animal origin may be ethically justified within a framework that considers ethics only within the human dimension but becomes difficult to justify when considered within the broader context of efficient use of resource and sustainable management of the ecosystem, especially the huge areas of natural grasslands and savannah (grasses, trees and shrubs). At present, much of this land has been degraded by overgrazing. However, well-managed pastoral and silvo-pastoral systems can improve the quality of the land as measured in terms of plant and animal diversity, soil quality, carbon sequestration and amenity value. Conservation grazing, using a stable population of suitably adapted ruminants involving a sensible programme of population control, can be an essential to this approach to sustainable land management, sustained, in part, through a policy of controlled culling of animals for human consumption. This can be more profitable and more humane than leaving them out to starve or be eaten by wolves.

Pollution of the soil and water with agricultural wastes from intensive livestock units is, I repeat, a case of too much potentially valuable fertiliser in the wrong place. In the case of pollution with nitrogenous materials, much of this arises from a non-renewable resource bought into the unit in the form of fertilisers and high-protein feed supplements, and disposed of at too high a concentration, too close to the factory farm. The core principle of organic farming is to ensure the maximum possible contribution, recycling, and conservation of resources derived from within the farm itself. In the short term, this can never generate yields to compare with production units that depend wholly or in large part on purchased, non-renewable resources. In the long term however, they offer the only truly sustainable option.

Currently, methane production from ruminants is estimated to contribute ∼10% to the planetary production of greenhouse gases (GHG). The current cattle population of the USA is (very approximately) 100 million animals, of which about 40 million are adult cows. It has been estimated that in the seventeenth century, before the arrival of Europeans bent on slaughter, the bison population of North America was ∼60 million. After adjusting for the fact that grazing animals produce more methane per unit of digestible energy than cattle fed on high concentrate rations, one can make a rough estimate that methane production from ruminants in North America is only about 20–25% higher than it was 300 years ago. If, as seems inevitable, we are compelled to reduce world production and consumption of meat and milk by 20–25%, then levels of methane production from ruminants should return to pre-industrial levels.

Moreover, as explained earlier, this assertion fails to consider the extent to which this effect may be mitigated by carbon sequestration, especially in situations where ruminants derive their sustenance entirely (or almost entirely) from permanent pastures. Well-managed grasslands can constitute a significant carbon sink, the extent of carbon sequestration depending on factors such as the intensity of grazing and the balance between grasses and legumes. The true impact of ruminants on climate change through the net production of greenhouse gases can only be determined by life-cycle analysis of the production and sequestration of greenhouse gases (CO_2 and CH_4) in different systems. While it is the case that estimates based on life-cycle analysis show that all current dairy systems make a positive net contribution to greenhouse gas production, it is far less than estimates based on CH_4 emissions alone and least of all when the contribution of pasture to the overall diet is greatest. Extensive systems of beef production from pasture are likely to be GHG neutral (as would have been the herds of prairie bison).

OPPORTUNITIES FOR CHANGE

We cannot escape the fact that our present rate of consumption of foods of animal origin is unsustainable. It is in our own interests to embark now on a strategic programme of change in livestock farming with similar aims to our current long-term programme to work toward net carbon balance. Indeed, the two strategies overlap within the same overriding, essential need: to restore the balance of nature. Unless we make some relatively painless changes to our lifestyle now, our children will have far more uncomfortable changes thrust upon them in the future. However, we will not (in sufficient numbers) do this of our own free will while the status quo remains so comfortable. We must be made to change. This will require a balanced menu of attractive carrots and humane applications of the stick.

This is a big subject. My brief is restricted to changes that can be achieved within the dairy industry. Any strategy for change must take account of, and give proper respect to, the needs of the consumers, the farmers, the environment and the cows. It must also plan for an absolute reduction in global milk production. This is counter to current economic thinking that continuous growth is essential to economic stability. In biological terms, this premise is, of course, an absurdity; well-expressed by David Attenborough who said "the only people who believe in continuous growth are economists and lunatics."

The prospect of new, greener, kinder approaches to milk production becomes more realistic when we reflect that the hyper intensive dairy industry in the affluent industrialised regions of today's world is not the norm, but a product of the last 50 years, an intense but unsustainable spike in the balance of nature. I rephrase my words at the outset: For most of recorded history the role of the family cow was to provide milk, work, fertiliser, fuel, clothing, and the occasional fatted calf for special occasions, while sustained by fibrous feeds that the family could not digest for themselves, usually from land that the family did not own. She was not competing with the family for food; she was an essential contributor to the harvest and she was valued accordingly. I am not suggesting that we should return to "the good old days," not least because for most people dependent on subsistence agriculture then and now throughout most of the underdeveloped world, days were and are not that good. What I am saying is that any future developments should incorporate all that is of value in new knowledge and technology but also ascribe proper value to the sources of this wealth, the cows and the land. Respect for cows may be a moral issue, respect for the land is a matter of survival. These principles apply equally throughout the dairy industry from the highly intensive >1,000-cow dairy units of Wisconsin to the dairy syndicates of India receiving and processing milk from multiple small famers, each with perhaps 2–5 cows.

Increased sustainability of food production systems depends on increasing the contribution of renewable as distinct from non-renewable resources. I have briefly described an elegant and comprehensive way to quantify these by way of "emergy" analysis (**Figure 1**). This makes it possible to estimate (with considerable uncertainty) an "Energy Sustainable Index" (ESI) for different systems. **Table 4** turns current agricultural economics on its head. Corn (maize), which ranks highest in terms of productivity (yield/ha) becomes the worst when measured in terms of sustainability. Beef cattle, sustained entirely from pasture are the least productive, but most sustainable. This is an extreme illustration of a general truth, which is that increased sustainability of food production from animals must be accompanied by a reduction in production. This has to be a good thing for the health and welfare of ourselves (the consumers), the cows and the living environment. It will however, cost more money and this may present serious problems for farmers and consumers, particularly those with least money to spend.

I have the good fortune to live in Somerset, classic cow country from time immemorial. The word "Somerset" describes the land of the Summer people, who brought their cattle down each summer to graze the coastal marshes, flooded in winter, but a reliable source of quality pasture throughout the driest of summers. **Table 2**, which shows that dairy cows can produce 40% more food energy for human consumption that they consume in terms of food that we could eat ourselves, is based on data taken from the feeding programme of my immediate neighbour, who grows over 60% of the feed for his cows on farm. The largest producers of yoghourt in the UK farm the Somerset grasslands to organic standards. A central tenet of their policy has not been to select their cows for milk production *per se* but for milk production from pasture, which inevitably means less milk per cow.

The dairy industry in New Zealand is almost entirely pasture based but presents cows (and their calves) many of the stresses associated with the most intensive indoor systems. Cows are expected to calve at 12-month intervals to synchronise peak lactation and peak grass supply. In 1960, 60% of the dairy herd were Jerseys. Thereafter genetic selection almost entirely favoured Holsteins based on criteria similar to those applied to Holsteins in the USA bred to live in barns. A selection index heavily weighted for milk yield was, in this environment, incompatible with maintaining high fertility at 12-month intervals. For some time, there was a policy to abort cows that were slow to conceive. Thankfully, this policy has largely been abandoned, reflecting a selection policy designed to place greater emphasis on fertility (Lembeye et al., 2021).

While the production of milk from grazed pasture can be an excellent example of good husbandry; farming the land for what it is best equipped to provide and selecting the cows best suited to this policy, it represents a small and diminishing sector of the international dairy industry. The greater challenge is to apply the principles of sustainability to the vast numbers of cows kept on large, industrialised units with little, if any access to pasture. In theory, it would be possible to provide a high proportion of feed from local renewable resources (e.g., organic grassland). In practise, short-term economics dictate that most producers will rely to a large extent on bought-in feed and fertiliser (NR). This leads to problems of waste disposal, especially N and P. The European Union has issued directives to limit emissions of N and P, reinforced by levies for exceeding defined limits. Dutch dairy farmers have responded to these directives by reducing the application of N and P fertilisers to grasslands and, in some cases reducing protein in concentrate rations. This application of the stick has reduced pollution problems at the "cost" of a small reduction in productivity as measured by lactation yield (Van Grinsven et al., 2016).

There are, at least in theory, several approaches to the reduction of methane emissions from rumen fermentation. As explained earlier, methane emission relative to milk production falls with increased milk yield and increased intake of starchy concentrates. Moreover, when cows are housed and fed on a formulated total mixed ration there is greater potential to reduce methane emissions through control of diet and manipulation of the ruminal microbiome (Bulumulla et al., 2017). It is also possible to reduce total emissions of GHG and other pollutants such as nitrates through improved manure management. Llonch et al. (2017) have reviewed the health and welfare consequences of alternative approaches. The bulk of the evidence suggests that methane production can be reduced by up to 50% by a combination of diet and the use of drugs such as ionophores. The very recent paper by Roque et al. (2021) suggests that natural sources of a trihalomethane (bromoform) may be more effective. However, any manipulation of the rumen population designed to depart from the "normal" carries the risk of reducing fermentation rate and thereby feed intake. The demonstration of heritable genetic differences in the rumen microbiome offers an alternative prospect of selecting a population of low methane producers (Wallace et al., 2019). It remains to be seen how this prospect may compare with the effects of diet and feed additives in terms of its potential impact on the environment, productivity, and welfare.

A particularly attractive solution to problems of methane emissions (and much else) is the development of Silvo-pastoral systems where cattle graze, browse, and relax within a parkland area of pasture, shrubs, and trees that act as shelters and carbon sinks (Cubbage et al., 2012; Vigne et al., 2013). In Brazil, for example, there are highly successful commercial silvo-pastoral systems (both beef and dairy), that generate income both from the cattle and the sale of tree biomass. A different but equally attractive example of ecologically sound diversification can be seen in the cork-oak parklands of Portugal grazed by the beautiful Mertolenga cattle. Income is generated from the sale of beef, corks for high-quality wines and tourists wishing to enjoy the natural environment. The cattle can select what to graze or browse and where to lie to their satisfaction (e.g., in sun or shade). In all but the most severe weather, they are comfortable and, above all, have freedom of choice.

These examples show that there are ways to produce green milk and meat from contented cows, but they are the exception. The more important question is how may we aspire to these aims within the great majority of industrialised high input/high output systems. Short-term economics that measure success simply in terms of profit margins will always favour the most intensive system. Some control over this can be achieved through imposition of penalties for environmental pollution, but greater progress can be achieved through a judicious selection of carrots. Happily, in recent years, public pressure for higher animal welfare standards and political pressure to mitigate environmental costs have started to move things in the right direction. One approach is to farm to organic standards set by the Soil Association[2] that require (e.g.) no use of artificial fertilisers and that a minimum of 60% of the ration should be based on fresh or conserved pasture. At present only 4% of dairy farms in the UK are organic. However, these farms are competing successfully because there is a niche market for organic milk. As I write, the average price for organic milk is about 40 p/l; conventional milk about 30 p/l. By contrast, oat milk, with a much lower nutritional value retails at about £1.40/l.

Public demand for high standards of cow welfare has had a greater impact than the demand for organic milk, probably because the financial cost to consumers has been relatively small. Thanks largely to public pressure for higher welfare standards, most dairy herds in UK now operate according the standards set by a Welfare Quality Assurance Scheme. Examples include the Red Tractor Scheme, RSPCA Assured (formerly Freedom Foods) and those established by competing supermarkets. All require monitoring by independent assessors to ensure compliance with the standards of the scheme. This is not the place to argue in detail about the relative merits of the different schemes. However, those operated by the supermarkets have had the largest uptake in terms of milk sold. This is an example of how competition within the free market can be a force for change. Supermarkets recognise a public demand for higher animal welfare standards, albeit somewhat price-elastic, and compete by including on their shelves products of animal origin, like milk and free-range eggs produced according to quality-assured high welfare standards. The aim is to attract customers to this supermarket on the basis of these assurances who then do the bulk of their food shopping in the same store. This allows the supermarket to pay a higher price for quality-assured milk, without significant effect on their overall profit margins.

While the incentives and penalties considered above are steps in the right direction, they fall far short of the changes needed to achieve the aim of "green milk," where "green" may be defined by net zero GHG emissions. This should be incorporated into the aims of the International Climate Commission and lead to government action enforced by law. The departure of the UK from the European Union has created the opportunity to rethink the agricultural support policy. It has been proposed that *all* agricultural subsidies should be redirected from support for food production toward support for public goods such as long-term management of soil and water resources, carbon sequestration, diversity of habitat and wildlife conservation. This would recognise that farmers are, by default, not only food suppliers but the most important direct custodians of the natural environment. This is a lofty aspiration. It remains to be seen how close we shall get to meeting this aim and whether the money involved will be sufficient to achieve significant improvement in environmental quality without bankrupting farmers in the process. In the specific context of green milk from contented cows, it has the potential to address two of the cows' greatest challenges, overwork and lack of choice. The stress of overwork can be reduced through feeding and breeding strategies designed to achieve the more robust cow, producing less milk per lactation, but with a longer, more comfortable, productive life. The problem of lack of choice can be addressed by ensuring that in any policy of environmental enrichment for the public good, the word "public" should embrace the cows.

AUTHOR CONTRIBUTIONS

JW performed the literature review, analysed and interpreted the findings, and wrote the manuscript.

[2]Soil Association Organic Standards. www.soilassociation.org.

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Consumers' Opinions and Expectations of an "Ideal Chicken Farm" and Their Willingness to Purchase a Whole Chicken From This Farm

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As poultry production and consumption have increased in the last decade, so have consumers' concerns about intensified production methods and the impacts they have on animal welfare. At the same time, poultry consumption has increased and enjoys great popularity. Also, a shift in consumers' consumption behavior can be observed as nowadays most consumers purchase chicken cuts, especially breast filets, rather than whole animals, mostly due to convenience and taste. Although consumer concerns have increased, market shares of alternative poultry products, i.e., those that are produced under higher standards compared to conventional products, remain comparably low. One of the main reasons are the large differences in prices. The higher prices for alternative chicken products such as organic result partly from increased production costs on farm level. Besides, consumer preferences for chicken cuts intensify cost differences. While alternative chicken breasts (e.g., organically produced) might be valued by some consumers, other cuts such as wings or thighs are not and are therefore sent into the conventional market. In these cases, the breasts need to remunerate all additional costs. Analyzing consumers' concerns about production methods and learning about consumers' obstacles to buy whole chickens might offer farmers greater possibilities to succeed in alternative markets. Therefore, the purpose of this study was to gain insights into consumers' chicken consumption behaviors, how consumers imagine an ideal chicken farm and whether they would be willing to purchase a whole chicken from this ideal farm. Three focus group discussions (total $n = 30$) with German consumers were held online in June 2020. The results show that participants associate the ideal chicken farm with four main characteristics: good husbandry system, positive economic impact for the farmer, high transparency, and proximate location of the farm in the same geographical region. However, willingness to purchase a whole chicken, even from the ideal farm, remains low due to mainly convenience reasons and daily routines.

Keywords: animal welfare, poultry production, consumer preference, whole chicken, chicken cuts, focus groups

INTRODUCTION

The poultry meat sector has grown worldwide for years, focused on indoor environments and automated production systems and processes (Fraser, 2008). In Germany, more than 620 million broilers have been slaughtered in 2019 (Statistisches Bundesamt, 2021). As poultry production grows, worldwide consumption of poultry meat also rises. In Germany, although meat consumption has decreased ∼2.2 kg per capita between 2009 and 2019 (Statista, 2020), poultry consumption has increased by 4.12 kg per capita in the last decade (Bundesinformationszentrum Landwirtschaft, 2020). This increase in poultry consumption has been related to consumers' association of this type of meat with a healthier diet (less fat content) when compared to other types of meat, particularly red meat (Kennedy et al., 2004; Spiller et al., 2010).

In production, also animal genetics changed and chickens have been selected based on their performance to obtain a greater muscular growth in a short period of time. Before the industrialization of poultry production took place, a broiler (i.e., chicken for meat production) needed around 120 days to reach a weight of 1.5 kg while nowadays this is achieved in 30 days only [Bundesanstalt für Landwirtschaft und Ernährung (BLE), 2020].

Moreover, with these intensified production schemes farm structures changed. The number of small poultry farms (<10,000 animals) in Germany decreased significantly from 1999 to 2016 while the number of big poultry farms (>50,000 animals) increased [Bundesanstalt für Landwirtschaft und Ernährung (BLE), 2020]. These big farms account today for 80% of all broilers produced in Germany [Bundesanstalt für Landwirtschaft und Ernährung (BLE), 2020]. However, along with these production schemes, public criticism, and concerns toward farm animal welfare in these systems have grown (Vanhonacker et al., 2008; Martelli, 2009; Nocella et al., 2010; De Jonge and van Trijp, 2013), particularly for broiler production and laying hens (Verbeke and Viaene, 2000; Martelli, 2009; Vanhonacker and Verbeke, 2009; Heng et al., 2013). Among the concerns related to the welfare of broilers are high stocking densities in barns (Halle and Sandilands, 2006), a lack of outdoor access (Busch and Spiller, 2018), and leg weakness due to the lack of activity (Bessel, 2006). In laying hens, the killing of day-old male chicks (Brümmer et al., 2017; Busse et al., 2019), and beak trimming (Heng et al., 2013) are additional sources of concern. Further, the use of antibiotics in animal production is negatively perceived by consumers due to their association with antibiotic resistances and residuals causing health problems in humans (Bernard et al., 2005; Yang et al., 2009; Busch et al., 2020).

As the term "animal welfare" has become increasingly debated by the public (Deemer and Lobao, 2011; Lagerkvist and Hess, 2011) and public criticism and concerns regarding the abovementioned topics have increased, it is necessary to understand what regular consumers (i.e., not experts on the topic) consider ideal characteristics of a farm where chickens are reared for human consumption. Although literature shows that animal welfare and type of husbandry system are important attributes for consumers (Tonsor et al., 2009; Vanhonacker and Verbeke, 2014), it is still unknown whether other characteristics are also of relevance to consumers when imagining an "ideal" production method. Although there has been research related to ideal pig (Sato et al., 2017), beef (Kühl et al., 2020), and dairy (Cardoso et al., 2016) farms, to the best of our knowledge there is still a gap regarding chicken farms.

Consumers buying decisions for chicken meat are impacted by several aspects, depending on the information available. Price is certainly among the most important attributes when making buying decisions for meat (Clark et al., 2017; Escobedo del Bosque et al., 2021) but animal welfare attributes are of importance, too. Increased willingness-to-pay for broiler chicken with higher welfare levels is generally given but is lower compared to other animal products such as dairy (Clark et al., 2017). People with higher levels of animal welfare concerns have found to eat less animal products and to pay more attention to welfare labels when buying animal products (Clark et al., 2016). Feed and product origin also influence buying decisions in broiler chicken and are of higher importance compared to breeds (Escobedo del Bosque et al., 2021).

Besides animal welfare concerns and an increase in consumption quantity, the intensive production methods of the poultry industry also allowed for a change in consumers' consumption behavior of poultry. Nowadays, it is possible for consumers to purchase only those cuts which they desire, e.g., breast filets, instead of whole animals. This increasing trend of purchasing specific chicken cuts (e.g., breast filets, thighs) (Birzele and Stetter, 2018) occurs since many consumers see convenience as the most important motive for purchasing specific cuts rather than a whole chicken (Kennedy et al., 2004; Ripoll et al., 2015). Additionally, consumers usually try to avoid associating meat with an animal (Kubberød et al., 2002) in order to feel less guilt (Te Velde et al., 2002; Hopkins and Dacey, 2008) or disgust (Kubberød et al., 2002; Hamilton, 2006; Hopkins and Dacey, 2008). Te Velde et al. (2002) suggest that the unrecognizability of the cuts (e.g., breast filets cannot be as easily recognized as a part of a chicken compared to a whole carcass) also influences the preference for cuts. Nonetheless, the changed genetics and the trend of purchasing only cuts has contributed to a decrease in the number of whole animals being sold (Birzele and Stetter, 2018) and therefore reducing the market segment of whole chicken consumption, which also impacts the production side. Consumers' increasing preference for chicken cuts also led to a shift from "short" (i.e., rearing for ∼33 days to achieve a weight of 1.5 kg) to "heavy" (i.e., rearing for ∼40 days to achieve a weight of 2 kg) fattening production methods in order to obtain larger and heavier cuts, particularly breasts (Bundschuh and Henning, 2016). Additionally, those cuts that are not consumed in European countries (i.e., wings, thighs, feet, organs) are exported to countries in Africa (e.g., South Africa, Benin, Ghana) and Asia (e.g., Saudi Arabia, Philippines, Hong Kong) (Bundschuh and Henning, 2016) often at low prices (Fourie, 2013; Banson et al., 2015; Bioland, 2020). For small producers of alternative markets in Germany, these developments are challenging. For them, exporting cuts is not economically viable. Selling whole chickens is therefore often an economic decision that is contrasting current consumer trends and challenging small and alternative production with e.g., increased farm animal welfare. The topic of consumers' willingness to purchase a whole chicken as well as the advantages and disadvantages of this product from consumers' perspectives has not been studied, to the best of our knowledge. Especially in combination with linking the consumption behavior to the impacts on the famer and the animals.

Since most consumers' knowledge about production systems in the poultry industry is limited (Erian and Phillips, 2017), the aim of this study was 2-fold: (1) to gain insights into how an ideal production of chicken meat looks like from a consumers' point of view; and, since it is known that consumers generally prefer cuts our aim was also (2) to assess the potential for marketing whole animals vs. cuts if the whole animal comes from such an ideal farm. The results of this study help understand consumer trade-offs between convenient consumption habits and support for preferred production methods. It indicates whether, and to what extent, consumers are willing to change consumption and preparation habits for the sake of production methods and farms that are in line with their values. In addition, results are relevant to chicken farmers in alternative (e.g., organic or animal welfare) markets in order to better understand what consumers expect from their practices and adapt these to their strategies in order to make chicken production more diverse and sustainable.

MATERIALS AND METHODS

To generate information addressing our research questions, we gathered qualitative data through online focus groups. As defined by Morgan (1996), focus groups are a research method to collect information on a preset topic through interactions in a group. According to this definition, focus groups have three main characteristics: collecting data, interaction in a group as source of data and an active role of the researcher in creating the group discussions. Focus groups allow creating an almost natural atmosphere that resembles a conversation setting with different opinions (Lamnek, 2005). This difference in opinions also allows participants to respond to and discuss with other participants and therefore generating further insights in the topic and reflect own views. As we did not know in advance all possible aspects that might contribute to an "ideal" broiler farm from a consumers' point of view as well as the drivers of consumer behavior regarding chicken cuts, we decided to choose focus groups as research mode. In addition to this exploratory approach we added some confirmatory elements in the focus group protocols for those cases in which respondents do not come up with topics for discussion by themselves.

In this study, all participants gave informed consent to take part in the study before the discussions started. This study was conducted in accordance with the Declaration of Helsinki, and the protocol including the leading interview questions for the focus groups was approved by the Ethics Committee of the University of Göttingen before data collection.

Three focus group discussions with German residents were held online in June 2020, using a virtual meeting room with audio and video sequences. Each discussion was scheduled for 90 min and moderated by a professional facilitator. Participants were recruited through an agency (Forester&Thelen Teststudio GmbH) in Hanover, Germany. The prerequisites for taking part in the discussions were consuming chicken meat at least once a month, consuming organic animal products and animal products with an animal welfare label at least once every 2 weeks or being responsible for cooking chicken for the family. The latter was the case for one of the participants in the group discussions (vegetarian that purchases and cooks chicken for the family), while all others were chicken eaters themselves. The age range for participants was set from 25 to 70 years. In each focus group discussion, 10 participants that live in Hanover, a city with ∼500,000 inhabitants in Northern Germany (and its suburban area) took part. Hanover was selected for the discussion groups as it is the capital of Lower Saxony, the state with the highest poultry production in Germany.

Accordingly, the questioning order was semi-structured in order to stay flexible within the discussion but also to have comparable results (Lamnek, 2005). The moderator followed the script of questions (see **Supplementary Table 1**) which was divided in five main parts and started with (1) a warm-up phase in which the rules for the discussion were explained and each participant introduced him/herself to the group (10 min). Next, in order to introduce participants to the topic of preferences for chicken meat, participants were asked in part (2) to describe their buying behavior of chicken meat (frequencies, point of purchases and determinants of buying decisions) and to rank the attributes that are of importance when buying chicken meat (20 min). These results will show a first glance at attributes that consumers value when purchasing chicken meat. Therefore, we then centered the discussion on our main research focus in parts 3 and 4. In part (3), participants were asked to describe an ideal chicken farm, according to their expectations. Included were questions about how relevant the farm is for consumers when purchasing chicken meat and which information about the farm would be wished to encounter at the point of sale (30 min). Part (4) of the focus group discussion was dedicated to the question whether participants prefer buying a whole chicken or cuts and for what occasions they buy each of these. In addition, participants were asked whether they would accept buying whole animals that originate from the ideal farm that they described before and how they rate the success of the approach of marketing whole animals. Advantages and disadvantages of marketing whole animals vs. cuts were discussed. Finally, the group discussions ended with (5) a closing question in which participants were asked to comment on the statement of eating less but better meat (25 min) followed by a summary and feedback section (5 min).

The discussions were recorded and transcribed by the recruiting agency and facilitated to the researchers. The transcripts were then revised and compared to the audio by two researchers independently. Next, the transcripts were analyzed following Knodel (1993) in two steps: (1) organizing and subdividing the data into segments: in this step, the collected data were looked at to determine where each topic started and ended; next, the data was divided into the four main questions asked in this study and (2) coding the material by determining criteria for converting it into analytically useful data. The coding of the transcripts was done in the following way (based on Knodel, 1993):

1. Development of codes that corresponded to each item in the discussion guideline: numerical codes were assigned to each research topic: 1 = "determinants of purchase," 2 = "ideal chicken farm," 3 = "whole animal vs. cuts," and 4 = "less but better meat." For each of these four major topics, subtopics were identified and statements were classified accordingly. For example, the topic of "whole chickens" was coded 3.1 and "cuts" was coded as 3.2 since they belonged to the topic coded with 3.
2. Creation of additional codes for topics that arose and were of special interest for the researchers: for instance, topics such as missing information from chicken meat packages or ideas on how to market whole chickens were mentioned. These were then allocated under a major topic and then coded differently than the subtopics included in the guidelines. For instance, "missing information" was coded 1.A as a subtopic of determinants of purchase (code 1).
3. Development of non-substantive codes that helped in the writing phase: statements that could be used as illustrative quotations when reporting the results of the study were marked with asterisks and italics font while subtopics or additional topics had numerical coding.

The coding of the material was done by two researchers independently, and then themes were compared and adjusted. Since different topics and subtopics were covered in this study, the analysis was done individually for each major category.

RESULTS

A total of 30 participants took part in the group discussions. The average age was 43 years, with the youngest participant being 25 and the oldest 64. A total of 15 men and 15 women participated and they were equally distributed in each session. In total, Six participants stated that they purchase and consume chicken meat more than once a week, while 13 participants do so only once a week and 11 once every 2 weeks.

Determinants of Purchase

At the beginning of each group discussion participants shared their purchase criteria regarding chicken meat. Fourteen participants purchased chicken meat in supermarkets 10 in discounter stores while only three participants purchased directly from a farmer, two in a weekly market, one in an organic shop and one purchased chicken meat online. When purchasing chicken meat, 63% of participants stated to mainly consider the price of the product. However, for some this meant "not the cheapest" product but included also the weighing with some quality criteria. When referring to a quality-price performance it was mentioned that "if the other criteria are met, I buy the cheapest." For 63% of participants, the type of husbandry system was as important as price Statements such as "having a good conscience" and "if I must eat it, then it (the chicken) should also had run around" were mentioned when referring to the importance of the type of husbandry system.

Further aspects that were of importance at the moment of purchase were: product origin (30%)—specifically regional origin, freshness (30%)—particularly the "best-before date," labels (30%), appearance (23%)—including color, paleness, leanness, and texture, and preparation (i.e., marinated or "pure") (23%). Other aspects that were, although less often, mentioned were: type of packaging (13%), amount of product (e.g., 500 g) (10%), cut (e.g., breast filets) (7%), brand (3%), sustainability (3%), age/size of the animal (3%), and spontaneous decision (3%).

Nonetheless, 20% of consumers stated to not find all necessary information they desire on the products at the point of sale. One participant said that "in the counter no answer about origin" could be given, referring to the salesperson not being able to provide information regarding the origin of the animal; two other participants agreed with this specific comment. Similarly, while discussing missing information, one participant mentioned that "feedstuff is missing" or "one can hardly recognize" referring to animal feedstuff. Additionally, when discussing the information on husbandry system labels participants stated "what is behind it?" showing that this type of information is not easily understandable for all consumers. Although the abovementioned attributes were of importance for participants when purchasing chicken meat, additional attributes that might also be of relevance—particularly those which are not mentioned on the product packaging, remain unknown. Therefore, we asked next, what an ideal chicken farm looks like for these participants.

The Ideal Chicken Farm

The ideal chicken farm, as described by participants in the focus group discussions, has four main characteristics. These categories were built from the different topics mentioned during the discussions. **Table 1** shows the topics discussed with regard to the ideal chicken farm and the counts how often the sub-topics have been mentioned by participants.

First of all, when consumers thought of the ideal husbandry system, free-ranging was mentioned by 30% of participants, while 20% of participants emphasized the importance of chickens walking on green fields with sufficient space. A smaller number of participants also mentioned "no mass production" (7%), "nice building" (3%), as well as the use of mobile chicken housing (7%) as part of the ideal farm.

Second, they mentioned the economic aspect for the farmer. Participants expected from the ideal chicken farm that the farmer practices circular farming, i.e., that all steps of production, from feed production to slaughtering, are done on the farm. In addition, three participants suggested that ideally the farm produces both, eggs and meat. Similarly, three participants mentioned that in an ideal scenario consumers pay more for the products, resulting in higher prices for the farmers; when referring to this topic one participant stated that farmers "do not participate in the price war and do not go bankrupt."

The third aspect that constituted an ideal chicken farm is transparency of production and species-appropriate conditions for the animals. While 14 participants mentioned transparency as an important part of the ideal farm, only a few gave specific examples of what they expected. For instance, two

TABLE 1 | Topics and sub-topics built from the group discussions of the "ideal chicken farm."

Topic	Sub-topic	Number of mentions
Husbandry	Free-range	9
	Green fields	6
	Space	3
	Weather protection	1
	No mass production	2
	Large farm	1
	Nice building	1
	Mobile housing	2
	Tents	1
Economics and production	Development of the farm	1
	Pre-ordering of meat	2
	Idyllic farm	2
	Cycle farming	12
	Utilization of everything	1
	Fair pricing	3
	Production of egg and meat	3
Animals and their lives	Balanced nutrition	3
	Species-appropriate behavior	12
	No shredding of chicks	4
	Animals live longer	1
	Transparency	14
	No antibiotics	2
	No growth hormones	1
	Antibiotics only when needed	3
Origin	From the region	5
	Regional feedstuff	8
	Farmers grow their own feedstuff	2
Definition of regional	Radius 50 km	3
	Lower Saxony	5
	Region of Hanover	2
	Under 100 km	3
	Germany	1
	Production not possible everywhere	1
	Nord Germany	1
	200 km	1
	300 km	1
	200–300 km	1
Farm size	Small	12
	Irrelevant if animals have enough space	7
	Irrelevant	6
	No mass production	3

participants stated to want information about the farm with "realistic pictures" on a website and to be accessible through Quick Response (QR) codes on the products. Participants were also interested in seeing how and where the animals are raised; statements such as "petting chickens and feeding them yourself," "know the farm owners, see where the animals live," and "guided tour for children" were mentioned by four participants as ways to see how the farm works. Regarding transparency, two participants also mentioned "where you can see feeding conditions" as an important aspect of the ideal chicken farm. Additionally, two participants wished to know how much space chickens have while 12 of them wanted to see that the animals are reared in a species-appropriate way. The use of antibiotics was mentioned, however the opinion seemed divided into two groups: the first wished no antibiotics in the production at all and the other group agreed with the application of antibiotics only when necessary. With regard to size of the farms, perceptions differed: 40% of participants clearly expected an ideal farm to be rather small, whereas 23% stated being indifferent on farm size as long as chickens are kept and treated in a good way or "if there is enough staff and space." For instance, first participant mentioned that even on an ideal farm "there can be 39,000 animals, but in small groups and kept in such a way that they get along."

As a fourth point, participants wanted the ideal farm to be located in their region in order to reduce transportation distances. Additionally, 27% of participants mentioned that the ideal chicken farm should use regional feedstuff, preferably grown on the same farm. Although most participants were not aware of which type of feedstuff is fed to chickens, 23% of participants highlighted that the feedstuff should "not be imported," "not come from South America," and "not be genetically modified." The question of what regional production means was also discussed. It was clear that consumers have different ideas: for 30%, regional was measured in a radius of 50, 100, 200, 300 km around place of residence while for others it meant a city (Hanover) (7%), a state (Lower Saxony) (17%), or even a country (Germany) (3%). As consumers have different ideas about what regional production means, one participant suggested indicating the distance between farm and point of sale on the product, which was supported by other participants in the group.

When consumers were asked to state, if they take all of the above mentioned aspects into account when purchasing chicken meat, only two claimed to do so while 10 stated that they only focus on one or two points (e.g., origin, animal husbandry).

Whole Chicken vs. Cuts

A higher share of participants (43%) in the focus groups stated to buy chicken cuts (e.g., breast, thighs, wings) instead of whole chickens. The 33% of participants who purchase whole chickens stated to do so mainly for special occasions like barbecue, when guests are visiting or when more time for cooking is available (e.g., weekend or holidays). The high time requirement was mentioned by six participants as the main disadvantage associated with the preparation of whole chickens. Additional obstacles of buying whole chickens were the need of "specific tools" such as cutting scissors, the large quantity of chicken meat that needs to be eaten and the problems associated to storing this large amount of meat. Among the advantages of purchasing a whole chicken "less food waste," "better taste," and quality ("I see what I purchase") were mentioned by eight participants.

On the other hand, four participants stated that the main advantage of cooking chicken cuts was also less food waste on the household level as well as the ability to cook the desired amount of meat rapidly. Additional advantages such as less workload, less time needed and easy recognition of quality were mentioned by six participants. The main disadvantages of cuts, mentioned by three participants, were seen in more food waste in the chicken industry as well as the lack of sustainability when purchasing only chicken breasts.

When confronted with the idea that the ideal chicken farm only sells whole broilers, the groups' opinions were again divided. Three participants said it was a bad idea. Three participants stated that the "idea is good but I would not do it" and one that it was "unrealistic" since some households consist of only one or two members. On the other hand, five participants thought it was a good idea and would purchase the product; however, three participants who would purchase the whole chicken stated to still prefer buying cuts (i.e., purchasing the whole chicken disassembled).

In order to generate demand for a whole chicken from this ideal farm, three participants stated that if the positive aspects of production (e.g., sustainability, animal welfare, regional production) were highlighted this could be possible. Other ideas that were mentioned to generate demand for this product included: "associate a whole chicken with an occasion, for example a Sunday grill," to reach a compromise by selling half chickens, to sell these whole animals to grillers, "education—explain good criteria for a whole chicken," to sell in one particular supermarket store and "he must make himself known there" (he refers to the farmer).

Finally, participants discussed under which circumstances their willingness to purchase a whole chicken would increase. Three participants suggested that including recipes on how to prepare the chicken would increase their interest. The idea of farmers offering a "package" with a "perfect meal" (i.e., vegetables to accompany a specific dish) was also suggested by one participant. When asked about purchasing smaller breeds which result in smaller animals three participants thought it was a good idea and could be an option to increase consumer interest since the amount of meat would be less and therefore more adequate for smaller households. However, three others argued against this point by saying "if I have to do the cutting, I prefer to have more," and one mentioned that it is not a good idea if it costs more.

Eating Less but Better Meat?

Finally, when responding to the statement eating "less but better meat," 13 participants thought this was a good idea, whereas six others asked why not eating "more better meat" or "paying more for meat," denying the reduction component of the statement. The question of what better meat meant was answered with higher quality (20%), animal friendliness (7%), and regional production (7%) as quality indicators by participants.

DISCUSSION

The Ideal Chicken Farm

When thinking about the ideal chicken farm, participants in our study mentioned a good husbandry system including free-ranging as an important criterion. This is in line with what others found (Martínez Michel et al., 2011), also holding true for pigs (Weible et al., 2016; Sato et al., 2017) and dairy cows (Cardoso et al., 2016). Outdoor access is often acting as a key indicator for an animal welfare friendly system in the consumers' perception (Busch and Spiller, 2018). Husbandry system has also been mentioned as important for making purchase decisions by participants in the focus groups (together with price). The importance of animal husbandry systems could be a consequence of the negative associations consumers have with animal rearing (Te Velde et al., 2002; Weible et al., 2016), mostly gathered from different sources such as television, newspapers, stories heard from other people, or visiting farms (Te Velde et al., 2002; Tonsor and Olynk, 2011; Weible et al., 2016; Erian and Phillips, 2017). Since consumers' definitions of animal welfare are usually different than farmers' definitions (Te Velde et al., 2002), it is important to understand what consumers expect. Consumers usually focus on housing conditions since these are strongly associated to the ability of animals to express their behavior, resembling their natural environment (Van Poucke et al., 2006; Sato et al., 2017). Consumers usually associate housing conditions to animal welfare (Sato et al., 2017; Vigors, 2019), and when thinking about positive animal welfare they think of small farms with animals living outdoors in a natural environment (Vigors, 2019). However, products of animals with a more animal-friendly husbandry system usually come with a higher price associated to a lower stocking density, more feedstuff needed, more space needed, etc. (Bornett et al., 2003; Lusk and Norwood, 2011). Nonetheless, many consumers state that they are willing to pay a higher price for animal products produces under higher animal welfare standards (Vanhonacker and Verbeke, 2009; Napolitano et al., 2010; Nocella et al., 2010; Clark et al., 2017). Even so, there is still a gap between the attitude (i.e., concern for farm animal welfare) and the actual behavior (i.e., purchasing a product with higher animal welfare standards) (Te Velde et al., 2002; Vanhonacker et al., 2008; Vanhonacker and Verbeke, 2009). This suggests that although many consumers might have concerns about animal welfare and have intentions of purchasing products with higher animal welfare standards, they might actually not purchase the product due to different and diverse reasons, also including the weight of a higher price (De Jonge and van Trijp, 2013). This is discussed as attitude-behavior or consumer-citizen gap (e.g., Ajzen, 2005; Vermeir and Verbeke, 2006). Also farmers have expressed doubt in consumers' and retailers' willingness to pay a fair price that would cover their expenses for implementing higher animal welfare standards (Bock and van Huik, 2007) and participants in the research presented herein valued price as equally important than husbandry system. Price has already been identified as the most important attribute when buying meat by others (Clark et al., 2017). In the focus groups presented herein, price as a purchase determinant did not necessarily mean purchasing the cheapest product but considering the price quality ratio. In the case of broilers, studies reveal that many people are concerned about animal welfare but there is a lower willingness to pay for it compared to e.g., beef (Clark et al., 2017). This challenges the selling of alternative chicken products and emphasizes the importance of good communication and available information about product quality.

A lack of information and availability of information on husbandry and welfare conditions is also an important factor that has been mentioned by our study participants and acts as a barrier in buying according to welfare attitudes ad preferences. Only few countries have specific welfare labels or labeling of husbandry systems on products, making it hard for consumers to get information. In Germany, for example, the labeling of husbandry systems on conventional meat has only been introduced by some retailers into the market in 2019. Even in the latter case of Germany, the meaning of such labeling is not necessarily self-explaining for consumers—as it has been stated by study participants.

As part of the ideal chicken farm, participants consider important that farmers are paid fair prices for their products. Other studies have shown that consumers' desire to support (local) farmers (Chambers et al., 2007) and are in favor of paying farmers fair and higher prices for their products (Padel et al., 2010; Busch and Spiller, 2016). Additionally, on an ideal farm they expect farmers to carry out all production steps on farm, including slaughter and feed production. This is rarely the case since protein feedstuff, particularly soy for poultry production, is imported from countries outside the EU [De Visser et al., 2014; Deutscher Bauernverband (DBV), 2016]. Additionally, animals are born in hatcheries and then transported to fattening farms and animals are not slaughtered on farm but rather at a slaughterhouse. Moreover, the slaughter of animals also implicates much more work for farmers and, even more importantly, the need of facilities designed and legally approved for slaughter. Therefore, carrying out all steps of broiler production on farm seems not realistic for the large majority of farmers. A compromise for this could be the use of mobile slaughterhouses as consumers have expressed an interest and a higher willingness to pay for mobile slaughtered animals (Carlsson et al., 2007; Hoeksma et al., 2017).

As urbanization has grown, consumers' distancing from agriculture has also increased (Albersmeier and Spiller, 2008; Böhm et al., 2009; Olynk, 2012). This disconnect from agriculture along with food scandals, have generated a lack of trust in agricultural production systems (Kubberød et al., 2002; Spiller et al., 2010; Berk, 2012). This distancing and lack of trust have increased consumers' demand for transparency in production processes over the last few years (Olynk, 2012). Our results confirm this, as participants indicated transparency as an important part of an ideal chicken farm, particularly regarding how and where the animals are raised. Nowadays consumers can find meat products with labels related to different topics such as: organic and sometimes husbandry systems, regional origin, and animal welfare. Although these labeling schemes have aimed to inform consumers and increase transparency of the production methods (Olynk, 2012), they are not extensively available and, apart from the organic label, well-known. Many consumers do not trust the information, are confused and do not know what each label means, or feel like there is an overload of information (Martelli, 2009; Vanhonacker and Verbeke, 2014). This was also reflected in our discussions, where participants revealed that when purchasing chicken meat, the information available was not always clear to them. Additionally, not all information (e.g., region of production, husbandry system, farm size, animal welfare conditions) is made available, particularly when purchasing meat at the counter. This problem was also mentioned by participants in this study who revealed that when purchasing meat at the counter the employee could not answer questions regarding the animal origin. This suggests the need for a consistent, clearer, and more transparent communication system when marketing such products, including the training of sales persons.

Product origin (i.e., farm location for animal products) is of high importance to many consumers as geographical proximity is usually associated to a high product quality, including freshness and better taste (Chambers et al., 2007; Grebitus et al., 2013; Feldmann and Hamm, 2015). Preference for local agricultural products such as fruits and vegetables and products of animal origin has been previously tested in several studies (Zepeda and Leviten-Reid, 2004; Chambers et al., 2007; Brown et al., 2009; Grebitus et al., 2013; Marcoz et al., 2014). In this study, participants mentioned the preference for regional products in order to reduce transportation distances. The preference for regional products due to the environmental friendliness of the production process, including transportation has also been found in other studies (Brown et al., 2009; Yue and Tong, 2009). Although most consumers were not aware of what chickens eat or where the feedstuff comes from, participants in this study mentioned the use of regional feedstuff as part of the ideal chicken farm's process. However, little is known about consumers' preference for animal feedstuff, including its origin. A few studies (Wägeli et al., 2015; Profeta and Hamm, 2018) show that there is potential for animal products produced with local feed as consumers would be willing to pay more for such products. Although most participants in this study prefer "regional" products, the definition of regional is still very ambiguous as each participant had its own criteria. This difference in perception of what regional entails can be attributed to the lack of an official definition and regulation (Feldmann and Hamm, 2015). Indicating the distance between the farm and point of sale, as suggested by participants in this study, seems like an easy and understandable way for consumers to determine themselves whether they are purchasing a regional product or not. The preference for labeling products with specific distances in miles/kilometers has also been elicited by consumers in Grebitus et al. (2013), although such labeling might be challenging for producers selling into different channels.

Whole Chicken vs. Cuts

Consumers' readiness to purchase a whole chicken (rather than cuts) from their ideal farm was divided, although it was seen as a good idea in general. Consumers resisting purchasing a whole animal stated that the amount of meat and work was too much for a household with one or two people. Especially when prices are higher for these whole chickens, these consumers will not be willing to switch to buying whole chickens as price play a predominant role in the buying decision (Clark et al., 2017). Ripoll et al. (2015) and Kennedy et al. (2004) found that consumers' main motivation to purchase cuts was the convenience of these pieces which is in line with our findings. Participants' interest in a product's convenience shows that consumers' lifestyles play a big role in purchasing behavior, even though they might compromise the "ideal" production method. As participants in this study suggested, the demand for whole chickens from the "ideal chicken farm" could be increased by focusing on promoting the sustainability, animal welfare, and regional production aspects of the products. This might be a good strategy as some consumers exhibit an increased willingness-to-pay for these aspects (Janssen et al., 2016). However, Vanhonacker and Verbeke (2014) suggest that rather than highlighting the benefits of higher welfare products, informing consumers about the current practices and their disadvantages might be a more efficient way to market products. In the case of marketing whole chickens it remains unclear how increased prices would further decrease consumers' acceptance compared to buying the more convenient cuts.

Not all consumers know how to prepare a whole chicken. The inclusion of cooking recipes as well as instructions for cutting the whole chicken (or selling a whole chicken already cut) were seen as good motivators to increase consumer's willingness to purchase a whole chicken. The inclusion of cooking recipes could help farmers to sell their products as a greater involvement in preparing and cooking food usually leads to purchasing local products (Cranfield et al., 2012; Zepeda and Nie, 2012).

Due to the obstacle many consumers have in purchasing a whole animal, an alternative for small-scale farmers could be offering a whole animal but already cut into the different pieces. In this way, consumers can have a whole animal without losing the convenience of individual storage and preparation of individual cuts. This system is currently used by Crowdbutching GmbH, were the animals (e.g., cows, pigs, chickens) are slaughtered only when all pieces have been sold. In the case of chicken meat, their website (http://www.kaufeinhuhn.de) allows consumers to choose from different packages of either cuts (including breast filets, wings, thighs, and drumsticks) or whole animals (mainly used for soups). Others are also using similar systems in solidary agriculture. Although such system implies more work for farmers or slaughterhouses/cutting facilities (slaughter, disassembling/cutting, and packaging), it also provides an opportunity to attract consumers and expand markets.

Eating Less but Better Meat?

A high consumption, and therefore production, of meat is associated with environmental issues such as high greenhouse gas emissions, water and soil contamination and a loss of biodiversity (Deckers, 2010; De Vries and De Boer, 2010; Lesschen et al., 2011). However, only few consumers have an idea of the environmental impact of meat production and consumption, and they usually underestimate this impact (Macdiarmid et al., 2016; Hartmann and Siegrist, 2017). Accordingly, many consumers are not willing to reduce their meat consumption or substitute meat for other protein sources (Rothgerber, 2012; Macdiarmid et al., 2016; Hartmann and Siegrist, 2017). This behavior is justified with satisfaction-related (e.g., taste, satiety) or health-related (e.g., necessary for strong muscles, need for animal protein) arguments (Rothgerber, 2012; Macdiarmid et al., 2016). This is also reflected in our study, where only a third of participants agreed with the phrase "eat less but better meat," while the other participants questioned why "less meat" and not "more."

CONCLUSIONS

In this study, eliciting what consumers perceive an ideal chicken farm was the main research goal. Four main aspects could be found to be of importance for many: (1) husbandry systems with much space for the animals including free-ranging, (2) circular farming (all is done on the farm, from fodder production to slaughtering) with adequate remuneration of farmers for their efforts, (3) transparency about good animal conditions for consumers and (4) geographical proximity between place of production and consumption. In summing up these results, the "ideal" chicken farm from a consumer's point of view is quite different from common conventional production systems that usually produce intensively indoors, buy the animal feed that is internationally produced and traded, and sell the products into anonymous markets where consumer cannot easily trace the product back to single farms. Although these mentioned aspects constitute the ideal chicken farm, many participants only take a few attributes into account when making a purchase decision at the point of sale. This finding supports the phenomena frequently discussed as consumer-citizen or attitude-behavior gap. In order to let this gap shrink and to support consumers behaving according to their attitudes, there is a need for improved communication when selling products with improved production methods, especially improved welfare. The information needs to be available in an easy, recognizable and independent way at the point of sale. The strong preference for purchasing cuts instead of whole chickens might be a challenge for producers with high welfare and sustainability standards. In order to get their efforts remunerated, those farmers need to make all information and processes transparent and invest in good communication to highlight the advantages of their products. Nevertheless, market segments for whole chicken, although produced on an "ideal" farm might remain a niche segment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

CB, AR, AS, and GB: conceptualization and methodology. GB: validation. CB: formal analysis, resources, data curation, and visualization. CB, AR, and GB: investigation and writing—review and editing. CB and GB: writing—original draft preparation. AS: supervision and funding acquisition. CB and AS: project administration. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2021.682477/full#supplementary-material

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Data Driven Insight Into Fish Behaviour and Their Use for Precision Aquaculture

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Aquaculture, or the farmed production of fish and shellfish, has grown rapidly, from supplying just 7% of fish for human consumption in 1974 to more than half in 2016. This rapid expansion has led to the growth of Precision Aquaculture concept that aims to exploit data-driven management of fish production, thereby improving the farmer's ability to monitor, control, and document biological processes in farms. Fundamental to this paradigm is monitoring of environmental and animal processes within a cage, and processing those data toward farm insight using models and analytics. This paper presents an analysis of environmental and fish behaviour datasets collected at three salmon farms in Norway, Scotland, and Canada. Information on fish behaviour were collected using hydroacoustic sensors that sampled the vertical distribution of fish in a cage at high spatial and temporal resolution, while a network of environmental sensors characterised local site conditions. We present an analysis of the hydroacoustic datasets using AutoML (or automatic machine learning) tools that enables developers with limited data science expertise to train high-quality models specific to the data at hand. We demonstrate how AutoML pipelines can be readily applied to aquaculture datasets to interrogate the data and quantify the primary features that explains data variance. Results demonstrate that variables such as temperature, wind conditions, and hour-of-day were important drivers of fish motion at all sites. Further, there were distinct differences in factors that influenced in-cage variations driven by local variables such as water depth and ambient environmental conditions (particularly dissolved oxygen). The framework offers a transferable approach to interrogate fish behaviour within farm systems, and quantify differences between sites.

Keywords: machine learning, hydroacoustic, aquaculture, AutoML, IoT

1. INTRODUCTION

1.1. Background

Salmon fish farming started on an experimental level in the 1960s but became an industry in Norway and the UK in the 1980s, and in Chile in the 1990s (Laird, 1996). Global salmon production is currently circa 2.4 Million Tonnes per annum in 2018 (FAO, 2020) with a market value of approximately 16 billion euros (Planet Tracker, 2021). Current production is mainly concentrated in Norway, Chile, UK and Canada. The intensification of the salmon industry requires more specific knowledge of the use of feed and the control and response to the environment. The individual number of animals used in aquaculture has increased substantially over the last 3 decades. For example, only for Scottish aquaculture the number of fish transferred to sea increased from 25 million in 1990 to 47 million in 2018 (Marine Scotland Science, 2018).

The use of big data and models to guide production in the *agriculture* space is well established, with initial implementations of *precision agriculture*, or information-based management of agricultural production systems beginning in the 1980s. The fundamental approach reduces to leveraging data from disparate sources (satellite, sensor arrays, image data, etc.) to guide decision and apply treatment in the right place at the right time. While precision agriculture originated with crop productions, applications related to livestock farming subsequently blossomed. These generally related to leveraging various sensor technologies to monitor health and productivity of livestock. Examples include radio frequency identification (RFID) tags to identify cattle in computer-controlled feeders, milking robots that ease the work of dairy operators, and automatic milk feeders that customise the milk supplement for calves, measure body weight and body temperature and generate reports (Gebbers and Adamchuk, 2010).

Precision *aquaculture* on the other hand is a nascent management concept that requires adoption of technologies from both crop and livestock management systems. Namely, an effective aquaculture management system needs to understand the environmental conditions *and* the conditions of fish within that cage. Modern fish farms are comprised of cages with up to 200,000 fish. As farms are typically composed of 10–20 cages, and multiple farms are often co-located in a bay, the total number of individual fish is enormous. This precludes the direct translation of concepts from livestock farming, and in practise, precision aquaculture is a marriage of approaches developed for both precision livestock and grain cultivation, i.e., fish are not managed as individuals as are cows, yet are obviously more complex in management than plants (O'Donncha and Grant, 2019).

There has been extensive literature on observing, modelling and quantifying the environmental conditions of aquaculture systems to inform on aspects such as site carrying capacity (Ferreira et al., 2013), environmental impacts (Buschmann et al., 2006), and mitigation activities to reduce environmental footprint (Costa-Pierce, 2003). These class of studies contribute to the first pillar of precision aquaculture; of equal importance is monitoring and extracting insight on fish behaviour to inform operations. In this paper, we investigate fish behaviour within a cage using hydroacoustic sensors. These datasets provide high resolution estimates of fish motion at the biomass (or group) level, and allow inference on fish behaviour and implications for farm management. Such data and the information they provide can be fundamental to empowering precision aquaculture and data-driven decision making.

This paper presents an analysis of environmental and hydroacoustic data from three salmon farms in Norway, Scotland, and Canada. We use statistical and machine learning approaches to interrogate the primary environmental drivers of changes in fish behaviour (i.e., variations in vertical motion and distributions). The contributions of the paper are as follows:

- We describe the monitoring, collection, and statistical analysis of environmental and hydroacoustic datasets at three salmon farms with very different environmental and geographical characteristics.
- We outline an AutoML (or automatic machine learning) model development pipeline (data curation, preprocessing, and model setup) to train and deploy a machine learning model to forecast fish behaviour using a "no-code" paradigm.
- We present a framework to interrogate the trained model and extract insight into the environmental drivers of fish behaviour using explainable machine learning techniques. We discuss the results of the data driven interrogation of fish response against known drivers of fish behaviour from literature.

The objective of the paper was to develop a transferable approach to interrogate caged salmon behaviour and inform farm management. Results indicate that data-driven approaches have great promise to provide automated insight into fish behaviour, and the environmental conditions that influence that behavioural response. However, the analysis needs to be informed by domain expertise from ecology and fish welfare to allow a robust interpretation of results.

The paper is structured as follows: below, we present a detailed overview and literature review of fish behaviour and how information on fish movement and vertical distribution provides insight into behavioural and welfare aspects. Section 2 describes the study sites, introduces the machine learning approaches used, and outlines the data curation and analysis framework. Section 3 presents results from this study while finally, we outline conclusions from this research and makes recommendations for further work.

1.2. Behaviour

Many studies of fish behaviour are in the wild or under controlled laboratory conditions. Few of them have been done under farmed conditions mainly due to the challenges and multiple restrictions this production systems poses to the researchers (Johansson et al., 2006, 2014). However, a greater understanding of the role of fish behaviour as a key health and welfare indicator is essential to allow more autonomous monitoring of fish health. The importance of fish behaviour as a farm management tool has spurred interests in new technologies to monitor and infer behaviour such as sonar and video images or the use of artificial intelligence.

1.2.1. Salmon Aquaculture

Many different biological, environmental and social parameters influence the behaviour of salmon when farmed in sea cages. Parasites, such as sea lice, are a biological example that can cause behavioural changes to farmed salmon in order to combat infestation (Bui et al., 2016). Sea lice are concentrated near the surface, and methods to limit fish surface time have been a focus of farm mitigation activities. Salmon have been observed to prefer deeper depths once highly infested to avoid further infestation (Bui et al., 2016). Temperature and dissolved oxygen

(DO) are an example of environmental parameters that affect the behaviour of Atlantic salmon. For example, Atlantic salmon will distribute themselves according to preferred temperature range, 8–18°C, with changes in behaviour occurring above and below the threshold (Johansson et al., 2006, 2009; Oppedal et al., 2011). Similarly, DO is an important parameter affecting fish behaviour as concentrations below the optimal range cause physiological stress and related behavioural changes such as a reduction in feeding (Dempster et al., 2016; Oldham et al., 2018). Light intensity is another major contributor to fish behaviour by changing vertical distribution. During daylight hours, when light intensity is at its greatest, fish tend to swim deeper in net pens to avoid surface predators (Fernö et al., 1995; Oppedal et al., 2001). It has been hypothesised ascent toward the surface during nighttime is a photo-regulatory behaviour to maintain schooling as light fades. Furthermore, seasonal variation in light availability changes vertical distribution with winter swimming depths generally shallower than summer swimming depths (Juell and Westerberg, 1993; Oppedal et al., 2001). However, this diel seasonal pattern changes when surface mounted artificial lights are installed in net pens (Oppedal et al., 2001). Populations have also been observed to remain in the upper half of net pens, under normal stocking densities, to avoid large piscivorous fish present under net pens (Fernö et al., 1995; Juell and Fosseidengen, 2004; Johansson et al., 2006, 2009; Dempster et al., 2016; Føre et al., 2017). Additionally, hydrodynamic conditions, such as waves and currents, will affect vertical distribution with stronger waves encouraging fish toward the surface (Johannesen et al., 2020).

A group of fish that voluntarily remain together, or a shoal, will have social group behaviour (Martins et al., 2011). A shoal will adopt a polarised swimming pattern in order to minimise the possibility of collisions and by synchronising these patterns a shoal can be deemed a school (Oppedal et al., 2011). Within a school there are rules in which all individuals must follow, and deviations from these rules by one or a few individuals can result in a group reaction. As with any population, individuals within a school will react differently when placed under the same stressors. For example, one fish may be more motivated by feed and swim toward the surface while another may be content waiting for feed to fall. These individual differences can affect the behaviour of the whole of the shoal regarding the responses to environmental parameters or other external or internal stressors. The internal state of the fish is also an important parameter to consider to understand the fish behavioural responses (Castanheira et al., 2017; Damsgård et al., 2019). Internal states being the final behavioural decision maker for the animal to respond to external stimulae (Huntingford et al., 2011).

1.3. Related Work

Technology on farms has increased significantly in the past decade (Føre et al., 2018), and ongoing efforts focus on the improvement of fish welfare and optimisation of farm operations, e.g., minimising the waste of feed. Sensors such as real-time oxygen and temperature probes, or acoustic tracking of fish are becoming more common in fish farming. The use of hydroacoustic sensors to infer the behavioural response of the fish to the physical structure of the cage, aquaculture practises, and the external environment can be a highly reliable and non-invasive operational welfare indicator (OWI) (Martins et al., 2011; Damsgård et al., 2019). A key area of research for the industry is whether sensor observations such as these can be used to augment welfare indices, thereby reducing the necessity to collect cumbersome manual samples such as lice counts and gill health.

Traditional methods of *in situ* observations of fish behaviour include visual inspections of the fish through random sampling, and video cameras placed in the feed zone of cages (Føre et al., 2018). Visual inspections are difficult to achieve on large populations and can be stressful to the fish (Martins et al., 2011). Video cameras can help identify when feed falls below the depth of the camera indicating the fish within the cage are satiated. Although these videos provide real-time images, they only supply a small frame of the cage and are hindered by the challenge of capturing high quality images underwater. In order to study behaviour on the cage's population, a larger view of the cage is required. One suggested method of continuous observations includes a commercial system, CageEye (2021). CageEye is a hydroacoustic sensor which is placed in the cage and captures (in real-time) the relative density of fish in the water column. Previous studies (Juell et al., 1993; Lindem and Houari, 1993) have investigated the effectiveness of using CageEye to completely automate feeding by using the detection of fish depth as indication of appetite. However, the use of this technology has potential to be used to study fish behaviour and indirectly welfare.

The association between behaviour and welfare can be determined for Atlantic salmon by understanding abnormal behaviour as it is linked with stressors (Martins et al., 2011; Damsgård et al., 2019). Therefore, continuous monitoring of fish behaviour can provide a more comprehensive perception of environmental conditions and their effect on welfare. The description, classification, and understanding of fish movement, as well as the environmental stimuli responsible for that behaviour could become the foundation for the creation of an early-warning system of fish welfare. This early-warning system can trigger changes in aquaculture practises that result in improved welfare conditions for farmed fish.

There are numerous studies dedicated to using machine learning to characterise and manage animal behaviour in *agriculture* (Liakos et al., 2018). These include automated monitoring systems based on video camera (Matthews et al., 2017), and prediction of bovine weight trajectories based on historical data (Alonso et al., 2015). Faced with a more difficult monitoring environment, applications of machine learning to aquaculture have developed slower. Many studies have investigated how machine learning could improve ocean monitoring and forecasting either by mining large ocean datasets Gokaraju et al. (2011) or relating future conditions to historical observations (Wolff et al., 2020). More recently, the applications of machine learning and computer vision technology to aquaculture is receiving a lot of attention. Broadly these are applied across two categories: 1) pre-harvest and during cultivation, and 2) post-harvest (Saberioon et al., 2017). In the pre-harvest and cultivation stage, much of the research focuses on monitoring fish behaviour. A number of studies have demonstrated accurate monitoring of fish behaviour and trajectory (Kato et al., 2004; Pérez-Escudero et al., 2014), although these have been predominantly laboratory-based. Monitoring and optimising feeding activities using computer vision and machine learning is an active area of research (Atoum et al., 2014). However, these provide little information about behavioural dynamics during feeding and are still at an early stage of development (Oppedal et al., 2011; Saberioon et al., 2017). Saberioon et al. (2017) provides an excellent review of applications of computer vision and machine learning technologies to aquaculture.

2. MATERIALS AND METHODS

Hydroacoustic methods provide a proxy measure for density and distribution of marine animals in form of acoustic backscattering (Foote, 2009). The fundamental principle is based on emitting a signal of known type and power level from a transducer. As it encounters regions of the medium with differing properties, also called heterogeneities, the sound is generally redistributed, or scattered, in all directions. This makes possible detection of the scattered sound with transducer and suitable receiver electronics. Advantages linked to hydroacoustic sampling techniques include, high spatial and temporal resolution, autonomous long-term sampling duration, range (especially during poor visibility when visual-based methods tend to fail), and a non-invasive surveying approach (Scherelis et al., 2020). Given these advantages, hydroacoustics is increasingly used to characterise animal behaviour in the marine environment, and considered a promising system to improve management of aquaculture farms (Bjordal et al., 1993; Juell et al., 1993).

In this study, hydroacoustic data were collected by one of two sensors "CageEye" (Scherelis et al., 2020) or "Aquaculture Biomass Monitor" ABM (2020). Broadly speaking, processed hydroacoustic data generates two metrics: volume backscattering strength (S_v), is often considered as a proxy for fish biomass; while target strength (TS) is an acoustic measure of fish length (Simmonds and MacLennan, 2008). TS is a measure of the acoustic reflectivity of a fish, which varies depending on the presence of a swim bladder and on the size, behaviour, morphology, and physiology of the fish. These outputs can be used to generate estimates of fish density and biomass (Boswell et al., 2007) within a cage.

2.1. Study Sites

This study considers three salmon cage farms in Norway (NOR), Scotland (SCO), and Canada (CAN). For each site a number of environmental sensors were deployed monitoring a range of parameters, including temperature, DO, and current speed. These were complemented with weather data from *in-situ* weather stations or model generated reanalysis from IBM Environmental Intelligence Suite available through their public API IBM (2021b).

2.1.1. Norway Site

The Norwegian site at Røssøya Nord (coordinates: 67° 4.38′ N 13° 56.855′ E) is a commercial farming site owned by GIFAS in Gildeskl municipality (Nordland, Norway). The site has a mooring system with placements for 16 cages (cage circumference 90 m; maximum cage depth 27 m). Seven cages (circumference 90m, max depth 20 m) were stocked with 18G S1 smolt produced at Salten Smolt AS and Helgeland Smolt AS in August/September 2018. Smolt were transferred at an average weight of 61 g (Salten) and 122 g (Helgeland) and each cage was stocked with approximately 150,000 smolt. The fish density at stocking was 1.3–2.1 kg/m^3.

All the cages were fed on a standard commercial diet (EWOS Robust). Feed was delivered from the silos on the barge to the cages via an air-blowing system and into a rotor spreader at the cage surface. Feeding start time, feeding intensity, and number of meals were adjusted according to day-length, weather, and observations on fish behaviour by the site staff.

A designated cage was instrumented to explore environmental variations and fish distribution (hydroacoustic sampling) between 20/02/2019 and 800 is a numerical ocean 31/10/2019. During the experiment it was decided to change the fish stock since they were not growing optimally. Therefore, the stock were replaced with 50,000 fish supplied by Helgeland Smolt in July 2019. The fish density after the change in fish stock was 10.1 kg/m^3.

Environmental variables were measured by Aanderaa instruments (Xylem analytics, Norway). The variables measured were salinity, temperature, DO, as well as current speed and direction. Animal variables were monitored by an Aquaculture Biomass Monitor sensor, ABM (Biometrics AS, Norway) which consists of a split-beam sonar mounted on a buoy and it can detect over 50,000 fish per day. The sonar provided an estimate of total biomass, biomass distribution with depth, fish weight distribution and fish swimming speed. Fish position and distribution are reported hourly, while estimates of average fish size are returned at multi-day period. **Figure 1** presents a schematic of typical sensor configuration within a cage. The hydroacoustic sensor was installed beneath the cage looking upwards. It sampled at an angle of approximately 42° from horizontal. An environmental sensor was deployed at approximately mid-depth in the cage.

Since the environmental sensor deployment (May–July) did not cover the entire study period, we augmented environmental data with output from the NorKyst-800 ocean model (Albretsen et al., 2011). The NorKyst-800 is a numerical ocean modelling system deployed to simulate physical oceanography variables such as sea level, temperature, salinity and, currents for all coastal areas in Norway and adjacent seas. The model has a horizontal resolution of 800 m, 35 layers in the vertical, and can be downloaded from the OPeNDAP server provisioned by the Norwegian Meteorological Institute (Norwegian Meteorological Institute, 2021). The model provides a satisfactory representation of Norwegian off- and onshore dynamics but requires higher resolution to resolve the dynamics of most Norwegian fjords properly (Albretsen et al., 2011).

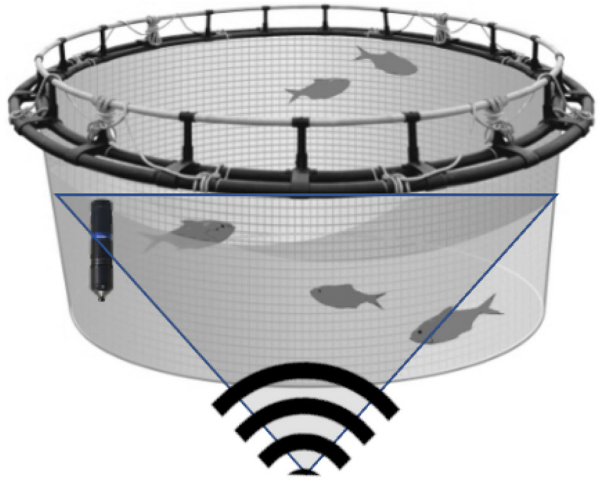

FIGURE 1 | Schematic of sensor configuration within a cage. The environmental sensor is denoted in the left hand side of cage with the exact location varying across sites (e.g., for Norway it was deployed at 5 m depth in centre of cage), while the hydroacoustic sensor and it's sampling domain is denoted beneath the cage (pointing upwards). Note, that the hydroacoustic sensor contains sampling "blind spots," especially toward the bottom of the cage which causes challenges in shallow sites where depth is limited. This figure represents an idealised representation of the deployment and naturally there are many practical considerations with regards sensor deployment (power source, ease of access, anchoring point, etc.).

2.1.2. Scotland Site

The salmon sea farm is located at Carness Bay, Orkney (coordinates: 59° 00.637′ N 02° 55.374′ W). This barge fed site includes a total of 12 × 100 m circumference cages and maximum net depth of 6 m. The total number of fish stocked at the end of January 2019 was approximately 230,000, with a stocking density of 5.1 kg/m^3. Fish stocked were 18S1 smolts, which were moved from Meil bay to Carness Bay on 5th February 2019. Each cage is stocked with approximately 20,000 salmon.

All the cages were fed on a standard commercial diet (Biomar, power extreme). Feed was distributed to the fish cage from the hulls on the barge via an air-blowing system. Feeding was controlled by AkvaConnect software (AkvaGroup AS). Feeding started at pre-determined times according to day length, most often with a meal duration of 30–60 min. The stock were monitored by camera and feeding intensity was adjusted accordingly. For example, should the fish exhibit poor feeding activity, the feeding intensity would be decreased or stopped in the affected cages. Adjustments in the feeding intensity were done daily according to requirement and light availability. As soon as there was enough light, the first meal started (lasting 30 min according to appetite), and fish were fed again after a pause of 4–6 h. A maximum of 2 meals were administered per day.

Cage 8 was instrumented to collect both environmental and animal variables i.e., variables concerning fish behaviour, growth and welfare data. Water temperature, DO, turbidity and salinity were measured for each cage daily by the site management staff. Cage 8 was monitored with two sensors to record DO and temperature: a handheld underwater wireless sensor (OxyGuard International, 2014) sampled daily from 08/01/2019 to 30/10/2019 and an *in-situ* Realtime Aquaculture sensor (Innovasea, 2021) sampled at 10-min intervals at a depth of 2.5 m from 13/11/2019 to 07/02/2020. As for the Norway site, due to the difficulty to collect continuous environmental data for the entire period, model data extracted from the Copernicus Marine Service model repository augmented sensor datasets. Temperature, DO, sea surface height (SSH), and current speed were extracted from the Atlantic North West Shelf model at the surface layer. Data is available at a 1.5 km (Tonani et al., 2019) horizontal resolution at hourly intervals and can be freely downloaded from the Copernicus portal. We compared sensor and model data for the period and since the overall trends were very similar, we augmented periods when sensor data were missing with Copernicus data. We noted that the model tended to overestimate magnitude of temperature, but since they captured the temporal variations, it served to adequately represent conditions for machine learning purposes. It's worth noting that the ML model is not affected by data magnitudes since data is normalised. Instead it learns how the predictand (label) varies in response to predictors (features). Using data from a physics model can be a pragmatic approach to handling missing values in environmental studies, thereby avoiding reliance on statistical imputation methods.

The relative biomass distribution within the cage was assessed using the beam sonar system, CageEye. The system in Orkney was made up of an echosounder and one transducer. The transducer was placed in the cage at a depth of approximately 5.5 m, and connected to the echosounder cabinet, which was placed on the cage ring and sent the data wirelessly to the base station at the feeding barge. The transducer was placed as deep as possible, looking up at most of the biomass of the cage. The transducer had two angles that they switch between, approximately 14 degrees (200 kHz) and 42 (50 kHz) degrees: this allowed one to get echogram recordings of both. It is important to note that this site did not have power at night, and consequently (since sensor did not have battery source), the acoustic sensor was switched off between 18:00 and 06:00.

2.1.3. Canada Site

The Canadian study site located in Saddle Island, Nova Scotia (coordinates: 44° 30.225′ N 64° 2.923′ W) is a commercially operated Atlantic salmon farm. The site had one column of 6 cages measuring 150 m circumference and a maximum depth of 11 m. Each cage contained approximately 60,000 fish with a stocking density of about 10 kg/m^3. Fish were fed twice daily, with the exact times dependent on daylight availability.

Each cage was equipped with two RealTime Aquaculture (Innovasea, 2021) probes deployed at 2 and 8 m depths. The probes measured temperature and DO, while an ADCP profiler sampling current speed was deployed in the northeast corner of the farm. Sea surface height was extracted from the Copernicus portal, in similar manner to the other two sites. Two of the cages were equipped with a CageEye sensor from 11/09/2019 to 30/10/2019. Each system consisted of three transducers, with two placed in opposite corners at 7 m depth, facing upwards,

TABLE 1 | Summary metrics for the three sites describing location, water depth, cage depth, average tidal range, number of fish per cage, and fish density.

	NOR	SCO	CAN
Latitude	67° 4.38′ N	59° 00.637′ N	44° 30.225′ N
Longitude	13° 56.855′ E	02° 55.374′ W	64° 2.923′ W
Water depth (m)	60	10	11
Cage depth (m)	27	6	11
Tidal range (m)	2.5	1.75	1.3
Fish per cage (-)	50–150,000	20,000	60,000
Fish density (kg/m^3)	1.3–10.1	5.1	10

Note that the fish stock were changed at the NOR site, hence we provide the range of values over the period.

and one near the surface, facing downwards. **Table 1** presents summary metrics on the three sites considered, while **Table 2** describes the data collected at sites. Data can be categorised along hydroacoustic and environmental sensor measured variables, and model or product data from ocean or weather model.

2.2. Machine Learning

Given sufficient data, machine learning (ML) models have the potential to successfully detect, quantify, and predict various phenomena in the geosciences. While physics-based modelling involves providing a set of inputs to a model which generates the corresponding outputs based on a non-linear mapping encoded from a set of governing equations, supervised machine learning (ML) instead learns the requisite mapping by being shown large number of corresponding inputs and outputs. In ML parlance, the model is trained by being shown a set of inputs (called features), and corresponding outputs (termed labels), from which it learns the prediction task—or in our case, we wish to predict the distribution of fish in a cage (as sampled by hydroacoustic sensor) based on a set of environmental measurements or features.

Classical works in machine learning and optimisation, introduced the "no free lunch" theorem Wolpert and Macready (1997), demonstrating that no single machine learning algorithm can be universally better than any other in all domains—variance tradeoff in effect, one must try multiple models and find one that works best for a particular problem. Selection of the most suitable algorithm and algorithmic settings is one of the most complex aspects of machine learning applications and highly dependent on user skill. An alternative approach leverages advanced *automatic machine learning* (AutoML) frameworks that aims to *learn how to learn* (Drori et al., 2018). AutoML systems uses a variety of techniques, such as differentiable programming, tree search, evolutionary algorithms, and Bayesian optimisation, to find the best machine learning pipelines for a given task and dataset (Drori et al., 2018). In this paper we applied IBM AutoAI (IBM, 2021a) to the data collected at the aquaculture sites. IBM AutoAI is a technology directed at automating the end-to-end AI Lifecycle, from data cleaning, to algorithm selection, and to model deployment and monitoring in the ML workflow (Wang et al., 2020).

As a benchmark, we compared results against a manually tuned machine learning model, namely Random Forest (RF). RF is one of the most popular machine learning models and has demonstrated excellent performance in complex prediction problems characterised by a large number of explanatory variables and nonlinear dynamics. RF is a classification and regression method based on the *aggregation* of a large number of decision trees. Decision trees are a conceptually simple yet powerful prediction tool that breaks down a dataset into smaller and smaller subsets while at the same time an associated decision tree is incrementally developed. The resulting intuitive pathway from explanatory variables to outcome serves to provide an easily interpretable model.

In RF Breiman (2001), each tree is a standard Classification or Regression Tree (CART) that uses what is termed node "impurity" as a splitting criterion and selects the splitting predictor from a randomly selected subset of predictors (the subset is different at each split). Each node in the regression tree corresponds to the average of the response within the subdomains of the features corresponding to that node. The node impurity gives a measure of how badly the observations at a given node fit the model. In regression trees this is typically measured by the residual sum of squares within that node. Each tree is constructed from a bootstrap sample drawn with replacement from the original data set, and the predictions of all trees are finally aggregated through majority voting (Boulesteix et al., 2012).

While RF is popular for its relatively good performance with little hyperparameter tuning (i.e., works well with the default values specified in the software library), as with all machine learning models it is necessary to consider the bias-variance tradeoff—the balance between a model that tracks the training data perfectly but does not generalise to new data and a model that is biassed or incapable of learning the training data characteristics. Some of the hyperparameters to tune include number of trees, maximum depth of each tree, number of features to consider when looking for the best split, and splitting criteria (Probst et al., 2019).

2.3. Model Setup and Training

Data preprocessing focused on creating a curated matrix of environmental and hydroacoustic datasets to allow statistical and machine learning interrogation of relationships. Important points to consider included outlier removal, time-averaging, imputation, data augmentation, and representation of temporal dependencies). **Figure 2** summarises the data processing workflow. The hydroacoustic sensor returns estimates of fish depth at sub-second frequency. This data point reports the location (relative to the sensor) of an individual (random) fish in the cage and is based on sensor detected change in medium (water vs. flesh). For a 6-month study, these generated about 45 GB of data. Data were first grouped into 1 m bins to represent the frequency of returns at different depth levels based on the *Echo Range (m)* measurement (i.e., number of individual fish in each 1 m bin). Measurements that are outside the extents of the cage were removed as outliers, and the remaining data were then time-averaged into hourly intervals. The binned data

TABLE 2 | Synopsis of data collection at the three sites summarising the environmental variables collected and the sampling periods, source of ocean model data (used to augment sensor data), and weather data source and variables.

	NOR	SCO	CAN
Environmental sensor	Aanederaa	Realtime Aquaculture	Realtime Aquaculture
Deployment dates	21/05/2019–02/10/2019	13/11/2019–04/02/2020	16/09/2019–16/11/2019
Variables	Temperature, DO, salinity, current speed	Temperature, DO	Temperature, DO, current speed
Hydroacoustic sensor	Aquaculture Biomass Monitor	CageEye	CageEye
Deployment dates	20/02/2019–31/10/2019	01/06/2019–29/09/2019	11/09/2019–30/10/2019
Ocean model data source	NorKyst-800	Copernicus Atlantic North West Shelf	Copernicus Global Ocean
Weather data source	IBM Environmental Intelligence Suite		
Weather variables	Wind speed, air temperature, solar radiation		

were depth-averaged to generate a time series vector that is amenable toward machine learning analysis. Equation 1 was used to compute the mean of grouped data.

$$\bar{x} = \frac{\sum fx}{\sum f} \tag{1}$$

where x refers to the midpoint of depth intervals and f denotes the frequency of fish in a given interval.

Data gaps or missing values were either imputed or removed: if the gap was less than 4 h, data were imputed using a nearest neighbour linear interpolation, while if gaps were greater than (or equal) 4 h, this portion was removed from analysis (i.e., both the environmental and hydroacoustic data were removed). Autoregressive features (i.e., values at previous points in time) are often informative for machine learning models. We generated these features using 3 h sliding window size (i.e., values at previous 1, 2, and 3 h). The resulting matrix is combined with environmental data, and time-aligned. We used our open-source packages, TSML (Palmes et al., 2020) and AutoMLPipeline (Palmes, 2020) for this preprocessing step. The code we used along with the data from the NOR site is available on Github at (O'Donncha and Palmes, 2021).

As part of the machine learning model setup, we investigated two configurations:

- All features: all available environmental variables together with sliding window values of fish location data were provided to the model. Each row represents the autoregressive features together with the date-time features (year, month, day hour, day of week, etc.), and environmental data. These features are time-aligned with the corresponding label (i.e., fish location) at the desired prediction window. We setup the problem as a 1-h ahead prediction using 3 h sliding window size (i.e., autoregressive at previous 3 h were included) with 1 h stride. Due to the lack of nighttime observations we did not implement this analysis at SCO site since the incomplete daily data can reduce the insight from autoregressive analysis. Instead, at SCO site, we only considered the configuration below.
- Selected features: to interrogate the strength of relationship or dependency between fish location and environmental conditions, a subset of features were provided to the model. The subset of environmental data were selected based on analysis of literature and the features identified as being important in the first experiment above. This configuration did not include sliding window values from previous timesteps which simplified the setup (missing data no longer had to be interpolated, instead those rows could be simply dropped). It also provides more flexibility when prescribing the prediction window since once could forecast whenever environmental data is available [e.g., one could leverage the Copernicus 10-day ahead ocean forecast data (Tonani et al., 2019) to make corresponding 10-day ahead predictions]. The selected features for this configuration were: temperature, DO, current speed and direction, wind speed and direction, sea surface height, and hour of day (described in **Table 2**).

The features (environmental data primarily) and label (fish location) data were split into two groups, to form the training-data set composed of 90% of the data, and the test-data set the remaining 10%. After preprocessing and hourly-averaging, the total number of data points available were 5,847, 1,574, and 840 data points for the NOR, SCO, and CAN study sites, respectively. The data were provided to the IBM AutoAI tool (IBM, 2021a) which automatically selected the optimal combination of algorithm, feature transformations, and calibration parameters (or hyperparameters) that minimised prediction error. Mean-squared-error (MSE) was selected as the loss function to optimise. We then used the trained machine learning model to interrogate how environmental data contributed to variations in fish location and behaviour. This can be considered the true goal of the machine learning implementation, and an accurate model simply served as a means to achieve this goal.

3. RESULTS

We collected data on the observed vertical distribution of relative intensity of fish biomass within a cage at three sites. The sites were geographically disparate and had distinct characteristics in terms of both the local environment, and the farm itself that influenced fish behaviour.

Figure 3 presents summary statistics for the NOR site: the top figure shows the centrepoint of the fish biomass over the duration of the study period, while bottom figure presents a box

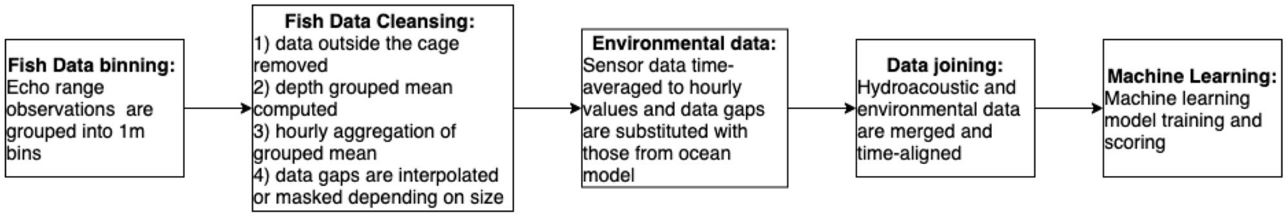

FIGURE 2 | Data processing workflow outlining the main steps of hydroacoustic data binning, data cleansing, environmental data processing, data merging, and finally machine learning model implementation.

plot elucidating hourly distribution every month. While each site exhibit unique characteristics, a number of common qualitative trends were shared, including:

- Each site demonstrated diurnal patterns to varying degrees (however the prominence of these varied across sites and at different times of year).
- Observations suggested a weak seasonal-scale pattern, with fish being at a higher position in the cage during summer months.
- A very pronounced preference for the upper portion of the cage that was independent of absolute depth. Generally fish tended to cluster in the upper one-third of the cage which can have significant implications for the density of fish in a cage (if the volume of the cage actually used by the fish is far less than the volume available).

These observations are interrogated in more detail in remainder of paper.

Figure 3 presents data from the Norwegian farm highlighting a number of noticeable trends. Firstly, despite the deep waters (approximately 60 m), and the large depth of the cage (27 m), fish tended to congregate in the upper third, and spent most of the time at depths of 3–9 m. The box plot does not show any pronounced daily pattern. It is worth noting that the northerly latitude of the site (67°N) means it is characterised by 24 h sunlight for most of the summer months which likely impeded the development of daily patterns during some of the period. Further, fish in the cage were changed between 26 and 29th July and new stock introduced, which naturally modifies recurrent patterns of behaviour. This may be the source of the more widely dispersed patterns of position evident in August, since the fish were newly introduced to the cage and conceptually displayed more chaotic behaviour patterns. Moving past these extenuating circumstances, the behaviours in September and October are possibly most indicative of typical cage-fish behaviour. These months are characterised by a weak diurnal pattern and fish congregating toward an ambient depth of about 9 m (or a third of the depth).

Figure 4 summarises information on fish distribution at the Scotland site. Both cage and water depth were significantly shallower at this farm, being 6 and 10 m, respectively. Naturally this affects the range that fish could travel and we see a quite tight clustering of average fish position between 1 and 2 m. Box plot indicates that fish sat within a tight half-metre cluster most of the time, with the box plot whiskers rarely extending outside this range. It's important to note that due to the CageEye transducer being placed inside the cage (because of the shallow water depth), some portion of fish in the cage will not be captured by the sensor. Hence, the degree of clustering is likely overestimated in this case. Results illustrate that average fish position in the cage tended to move closer to the surface during the summer period, likely influenced by warming surface temperatures. **Figure 5** includes temperature data reported at the Scotland site, illustrating warmer waters that peaked in early August before returning to moderate temperatures in September. The general trend of monthly variations in fish position, seem to follow these patterns, with July and September reporting comparable values for both temperatures and average fish positions. There was no clear diurnal pattern obvious in the data. It is important to note that due to lack of power during the night, data were not collected between the hours of 18:00 and 06:00. This naturally reduced the contribution of hour-of-day toward explaining the data.

Finally, **Figure 6** presents data from one of the cages at the CAN site. The CageEye sensor was deployed between 11/09/2019 and 30/10/2019, covering a period of large drop in temperature and reduction in daylight hours. A strong diurnal pattern was evident at this site with fish tending deeper in the cage during daylight hours. Due to the time of year the water column was not thermally stratified which may reduce the effects of temperature. While the cage depth is 10 m, the box plot illustrates that fish were generally clustered within a 2 m range and this cluster rarely goes deeper than 4 m in the cage, reflecting similar patterns to the other two sites.

Prior to more detailed statistical analysis of the data, one desires insight into the primary drivers that explains the observations. As discussed in section 2.3, machine learning models such as Random Forest provides a robust approach to efficiently explore multiple variables and associated response. We considered an analysis of the CageEye/ABM vertical distribution data from the three sites using IBM AutoAI (IBM, 2021a), automated machine learning tool. The data were preprocessed as described in section 2.3 and uploaded to the AutoAI website. The hydroacoustic data column was specified as training *labels*, and *features* were selected based on the particular experimental configuration (either "all features" or "selected features") using the AutoAI Graphical User Interface (GUI).

Our first experimental configuration ("all features" described in section 2.3) provided a wide range of environmental

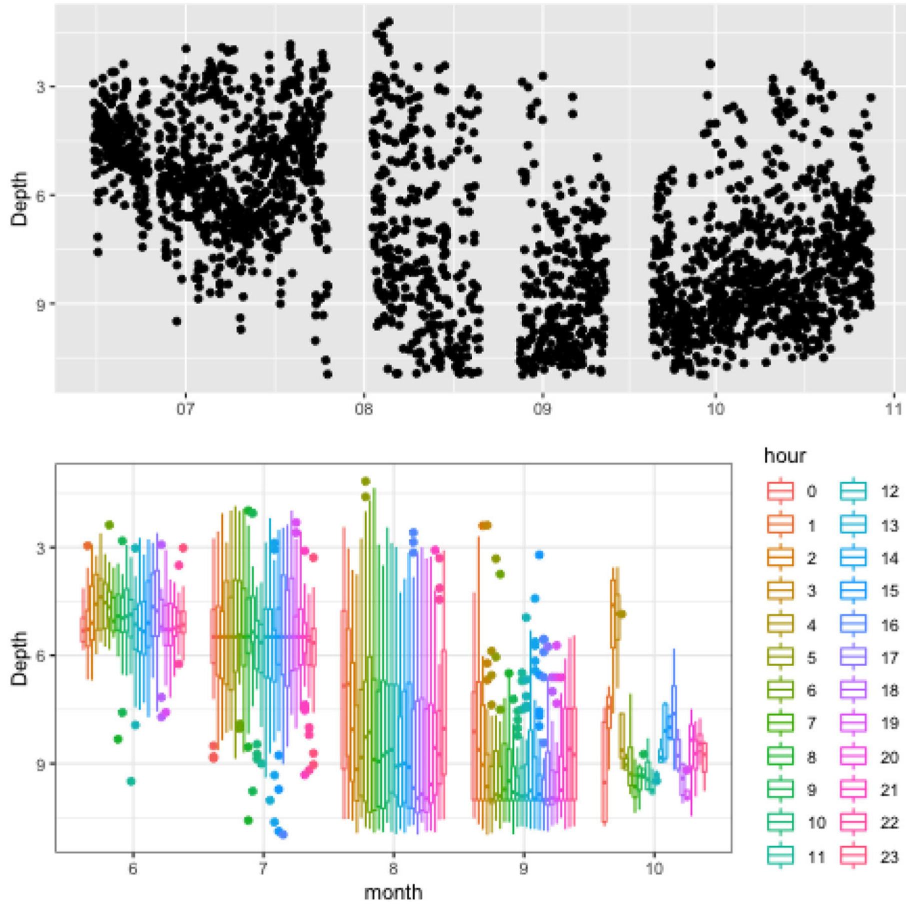

FIGURE 3 | Vertical distribution of fish in a cage at NOR farm illustrating the evolution of the centrepoint of fish biomass over the duration of study period (top) and boxplot of the data grouped into hourly intervals for each month (bottom). The box plot provides insight into distinct patterns developing in the data at different times with the color legend representing hour of day. Lines extending from the boxplot represent the range of data (i.e., minimum and maximum values), while the box section reports 25th percentile, median, and 75th percentile values. Filled circles represent outliers for the data.

(temperature, DO, current speed and direction, wind speed and direction, and sea surface height), temporal (hour of day, day of year), and autoregressive (measured fish position at 1, 2, and 3 h previously) variables as input (or features) to the model. The model was trained to make a 1-h-ahead prediction. Since the model implicitly learned to predict by learning the relationship between features and labels, we could then use the trained model to extract insight on how these features contributed to the model prediction. The resultant model demonstrate strong predictive skill reporting explained variance of 76, 81, and 75% for the NOR, SCO, and CAN sites, respectively. The relatively high correlation scores (equalling 0.87, 0.9, and 0.87, respectively) support the viability of using the model to explore the contribution that individual variables or features make toward prediction.

Figure 7 presents the *feature importance* of the supplied data to the response variable or model prediction at the CAN site (extracted from the AutoAI GUI). The feature importance measure computes the contribution or importance of each feature by calculating the increase of the model's prediction error after permuting the feature. A feature is "important" if permuting its values increases the model error, because the model relied on the feature for the prediction. A feature is "unimportant" if permuting its values keeps the model error unchanged, because the model ignored the feature for the prediction (Breiman, 2001).

Data from the CAN site provided some useful insight into salmon cage dynamics. As might be expected, autoregressive variables were a primary driver of fish behaviour. The most important feature is value at the previous timestamp ($x1$, denoting fish position 1 h previously) with $x2$ and $x3$ also contributing. Hour-of-day was the second most important feature which suggests that there was some diurnal pattern to the data that can be explained by this repeating feature. This information can serve to guide optimal feature selection for model development. Combined with domain knowledge on primary variables that influence fish behaviour (summarised in section 1.2), this information can lead to development of a more effective model. Selecting the most appropriate set of features is critical to maximising model performance, while from

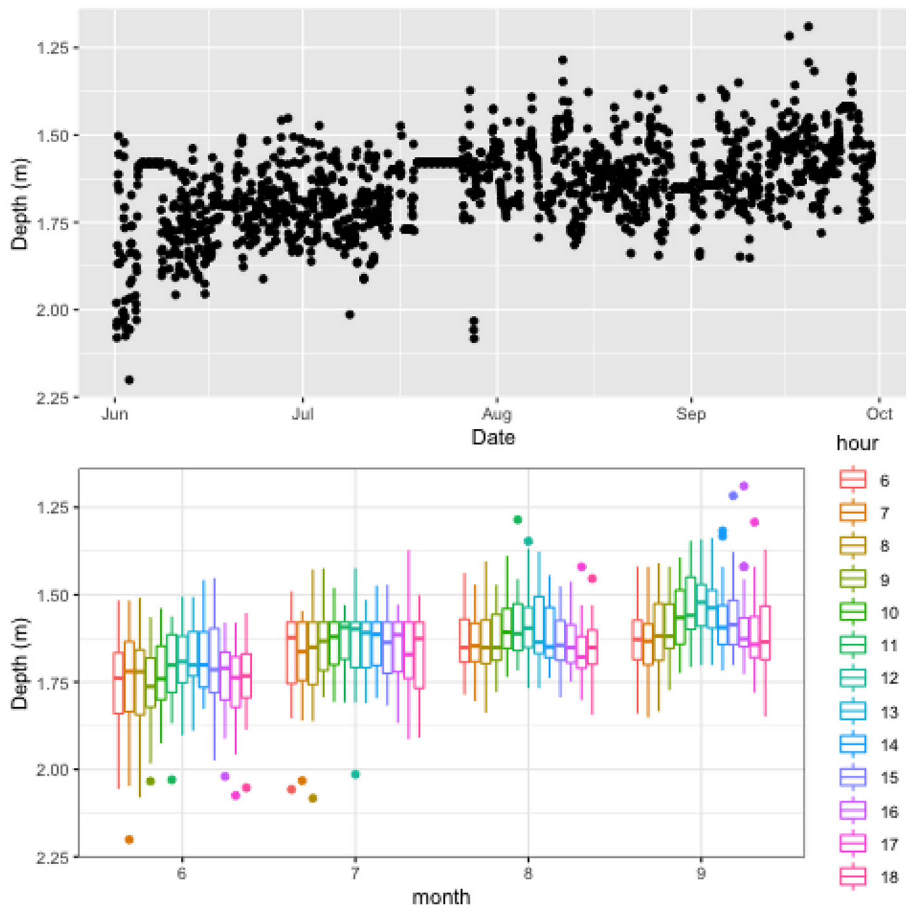

FIGURE 4 | Vertical distribution of fish in a cage at SCO farm illustrating the evolution of the centrepoint of fish biomass over the duration of study period (top) and boxplot of the data grouped into hourly intervals for each month (bottom). The box plot provides insight into distinct patterns developing in the data at different times with the color legend representing hour of day.Lines extending from the boxplot represent the range of data (i.e., minimum and maximum values), while the box section reports 25th percentile, median, and 75th percentile values. Filled circles represent outliers for the data. Note that the plots only include data between 06:00 and 18:00.

a practical point of view a model with less predictors may be more interpretable (Kuhn and Johnson, 2013).

Our second experimental setup involved a reduced set of features, namely: temperature, DO, current speed, wind speed, and salinity, together with hour-of-day. Choice of features were based on both feature importance reported in **Figure 7** and those suggested by literature. Naturally, the variance explained (or predictive skill) of the model dropped with the reduced feature set, but the analysis of feature importance or contributions can be more meaningful. The resultant model explained 59%, 64%, and 61% of variance for the NOR, SCO, and CAN sites, respectively, which represents a drop of 14–17% compared to the model with all features provided. This drop in predictive skill was balanced by an improvement in model *interpretability* and increased focus on pertinent variables (environmental conditions).

Figure 8 summarises model performance at the Canada site. It illustrates that the model captures data trends quite well reporting correlation score of 0.78. Visually, the model captures observed fish depth quite well considering the highly dynamic nature of the signal. In particular, trends in the data are adequately tracked and the model accurately replicates whether the fish move up or down in the cage in response to the provided model inputs. From a feature analysis perspective, this allows us to confidently interrogate results since we are primarily interested in variations in output rather than magnitude (i.e., changes of fish position in response to changes in environmental conditions rather than the magnitude of those changes) We used the model to understand variance explained by these drivers together with the feature importance of each. **Figure 9** presents the variable importance computed for the three locations in Norway, Scotland, and Canada.

While there were similarities in the drivers that influenced fish position at the three sites, pronounced variations existed based on the different geography and characteristics of each site. As suggested by both feature importance analysis and boxplot visualisation, time-of-day was a primary driver, particularly at the Canadian farm. This reflected the pronounced diurnal patterns that are visually evident in **Figures 3–6**, with the fish being

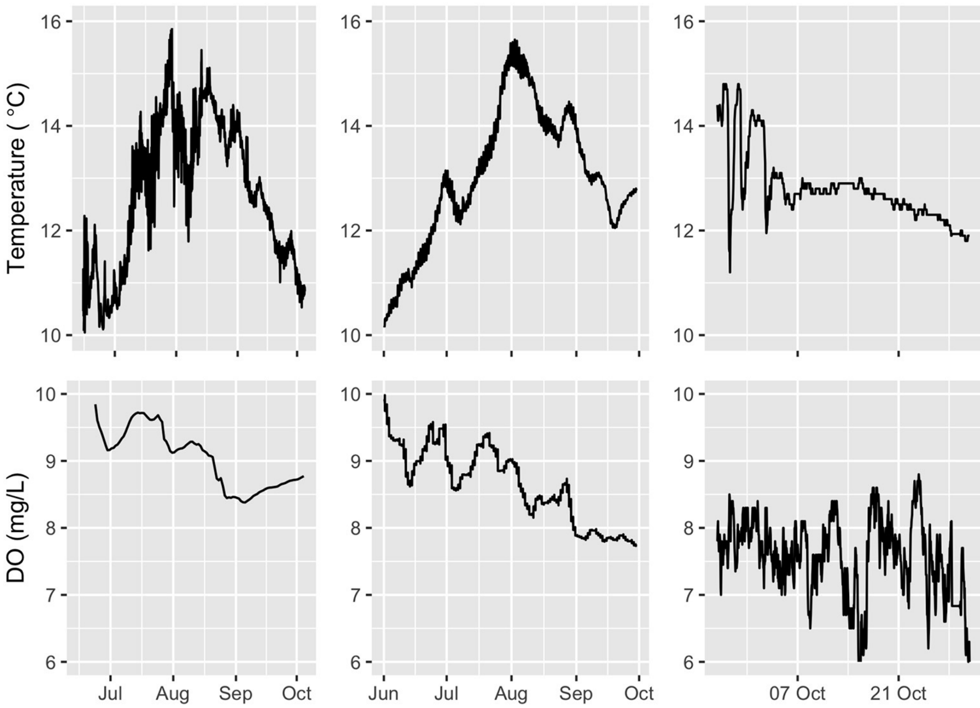

FIGURE 5 | Time series plot of temperature (top row), and DO (bottom row) reported at the three sites, NOR (left), SCO (middle), and CAN (right), respectively.

deeper in cage during daylight hours. It's worth noting that diurnal patterns were likely under represented in NOR and SCO data due to long summertime daylight hours and lack of nighttime observations, respectively. **Figure 10** presents a density plot of daytime and nighttime fish positions for both CAN and NOR (due to lack of nighttime observations, SCO was excluded). To remove the effects of long sunshine hours during June and July in NOR these 2 months were excluded from the plot. Results demonstrated a clear difference between daytime and nighttime behaviour for the CAN site and a similar but much less pronounced difference for the NOR site. In Canada, fish congregated at about 3.6 m depth and the spread around this was quite narrow during the day, while at night, fish were distributed more widely across the water column with a mean depth of 2.8 m. Similar trends were observed in Norway (although not as pronounced). The mean difference between daytime and nighttime positions were 0.52 m while fish were also more uniformly spread across the water column at night.

At all sites, physical oceanographic variables represented an important driver. Physical mixing by current speeds and wind forcing were particularly critical at the CAN site and three of the five most important variables represented physical stresses and mechanical mixing, namely current direction, wind direction, and wind speed, respectively (in order of influence). Wind stress did not represent an important driver of fish depth variance at the NOR site. This is likely due to the increased depth of cage and fish position serving to shelter from local surface dynamics. Interestingly, salinity was the primary driver of fish position at the NOR site which illustrates both fish sensitivities and local bay characteristics.

Figure 11 presents vertical profile of temperature and salinity at the site over the duration of the study period. Results illustrate a pronounced thermal stratification during the summer months, that breaks down into a well-mixed water column in spring and autumn. Variations of vertical salinity are more complex illustrating relatively low surface salinity values in September, which may be influenced by precipitation or freshwater runoff. Literature indicates that Atlantic salmon are influenced by salinity variations when younger than 3 months and during spawning periods, while indifferent to salinity at other times (Oppedal et al., 2011). The behavioural influence detected in this study may be a result of salmon expressing preference for lower salinity waters in spring, during the return migration period of salmon toward freshwater. However, **Figure 11** indicates that the vertical variation in salinity was relatively small, and additional study is necessary to understand the influence this may have on salmon variations.

While **Figures 7**, **9** provide insight into which features were important, we were interested in how the features influence the predicted outcome. A powerful approach to interrogate the variations of predictand in response to predictors are *accumulated local effects* (ALE) (Apley and Zhu, 2020). ALE quantifies the contributions of different predictors by considering the conditional probability or likelihood of changes to prediction. It has noted advantages in cases where multiple predictors are correlated and the effects are difficult to separate (which is naturally the case in ocean systems).

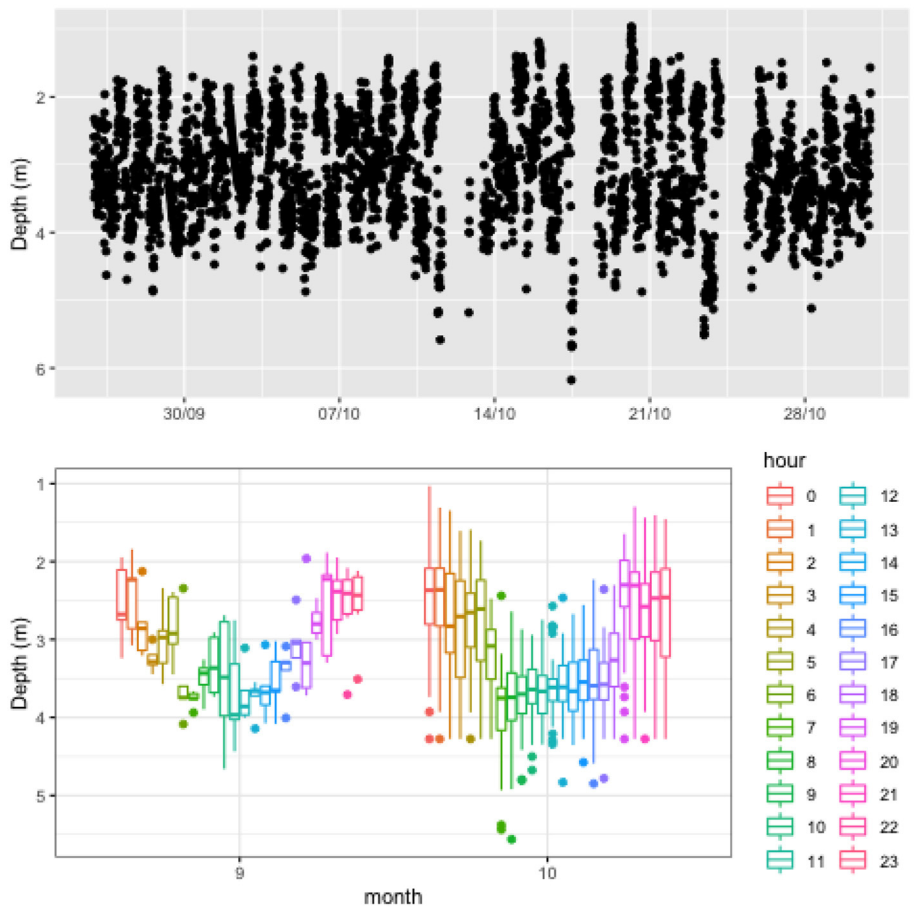

FIGURE 6 | Vertical distribution of fish in a cage at CAN farm illustrating the evolution of the centrepoint of fish biomass over the duration of study period **(top)** and boxplot of the data grouped into hourly intervals for each month **(bottom)**. The box plot provides insight into distinct patterns developing in the data at different times with the colour legend representing hour of day. Lines extending from the boxplot represent the range of data (i.e., minimum and maximum values), while the box section reports 25th percentile, median, and 75th percentile values. Filled circles represent outliers for the data.

Figure 12 presents the computed ALE for the CAN site for four variables, namely, temperature, DO, wind, and current speed to the response variable. ALE provides a quantitative way to show how the prediction (fish position) changes locally, when the feature (environmental variable) is varied. The marks on the x-axis indicates the distribution of the particular feature, showing how relevant a region is for interpretation (little or no points mean that we should not over-interpret this region). **Figure 12** allows for extraction of a number of pertinent observations on the data. The feature effects of temperature and oxygen suggest that "ambient" conditions had low importance (tends toward zero), while higher and lower values tends to trigger a response. Specifically, DO reports low importance when values were between 7–8 mgL^{-1}, while values outside this range invoke a large response by the model. It is worth noting that this large response by the fish is likely indicative of high-stress conditions. **Figure 5** plots time series of DO to illustrate the evolution at the site and localised periods when values dropped below 7 mgL^{-1}.

The contribution of wind and current speed to fish response were quite similar (as might be expected). Generally increased current speed invoked an increase in the model predicted value (i.e., fish were deeper in the cage). The plot suggests a linear relationship but is likely not enough data to draw confident conclusions on the exact relationship. This is amplified by the fact that the marks on the x-axis are quite sparse for higher values of wind and current speed indicating low number of observations for these conditions.

4. DISCUSSION AND CONCLUSIONS

The precision aquaculture concept aims to exploit data-driven management of fish production, thereby improving the farmer's ability to monitor, control and document biological processes in fish farms. The fundamental approach has been summarised as a series of steps, namely observe, interpret, decide, and act (Føre et al., 2018), that strives toward optimised operations of farms. Where precision aquaculture differs most prominently from its

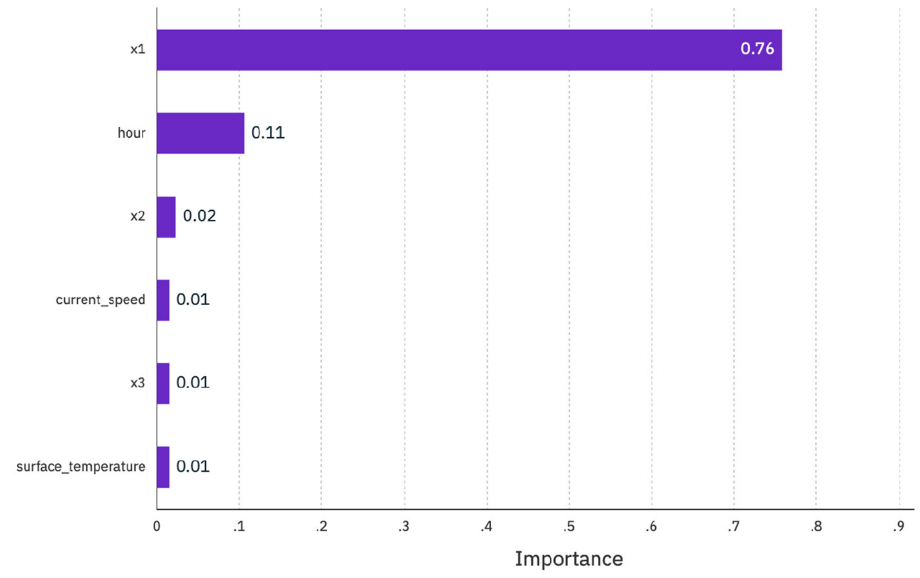

FIGURE 7 | Feature importance reported for first experimental configuration "all features" (section 2.3) at the CAN site. We provided all available data as features to the model (i.e., environmental data, temporal data, and autoregressive data or fish position at previous 3 h). The *y*-axis reports ranked list of features that contributed the most to variation in fish depth measurements, while the *x*-axis presents relative magnitude of those contributions. Ranking predictors in this manner can quickly help sift through large datasets and understand data trends (Kuhn and Johnson, 2013).

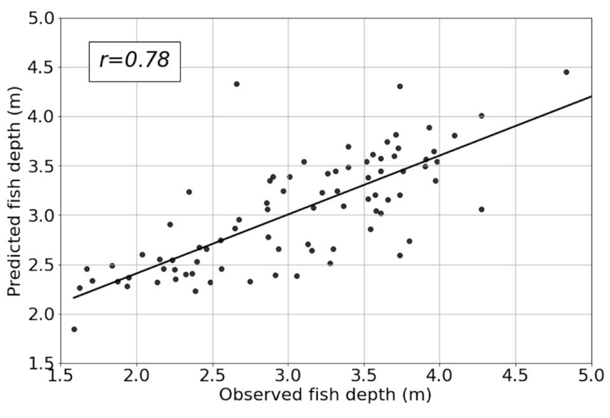

FIGURE 8 | Scatter plot of model predicted fish depth plotted against observed values for the CAN site. Inputs to the model are environmental data time-aligned with the target data, and hour of day to represent temporal variations (which **Figure 7** suggested to be an important contributor to fish motion).

sister industry, agriculture, is in the need for sensing of the ambient environment also (e.g., water temperature, oxygen)—a consideration that is less important in agriculture where animals can be housed (O'Donncha and Grant, 2019). In this paper, we adopted acoustic measurements of fish distribution to quantify how environmental conditions influence and modify behaviour.

Results demonstrated pronounced temporal variations in fish distribution as dictated by factors such as diurnal patterns, dynamics (currents and winds), and oxygen and temperature variations. Diurnal patterns driven by natural changes in light intensity were broadly similar across sites (although lack of nighttime data at SCO site limited interpretation for this site). Generally, fish occupied a deeper position in the cage during the day and were more tightly clustered; while at night, fish utilised more of the cage volume and were at a higher average position. These patterns were more pronounced at the CAN site, while the effect of longer daylight hours possibly ameliorate this effect during the June and July months at the NOR site. These diurnal patterns reflect what has been observed in the literature for salmon group response to natural light (Oppedal et al., 2011).

Analysis indicates that temperature was a primary contributor at the NOR and SCO site, while less influential at the CAN site (**Figure 9**). These results are partly influenced by the longer study period in these two sites that captured seasonal variation of temperature. **Figure 5** shows that temperature variation at the CAN site was between approximately 12–14°C compared to 10–16°C at the other two sites. Further, temperature in the warmer summer months exhibited pronounced stratification before returning to a well-mixed temperature profile in September and October. **Figure 11** presents vertical temperature profile for the NOR site, illustrating this summer stratification. Literature suggests that salmon prefer the highest available temperature (≤18°C) and avoid colder temperatures (Oppedal et al., 2001; Johansson et al., 2009). On the other hand, in reasonably homogeneous environments where temperature varies little with depth (such as CAN site during autumn), temperature is not expected to influence the vertical distribution of salmon (Oppedal et al., 2011). Hence for the sites studied here, one may expect active behavioural thermoregulation during the summer and not in other months where temperature varies little within the cage.

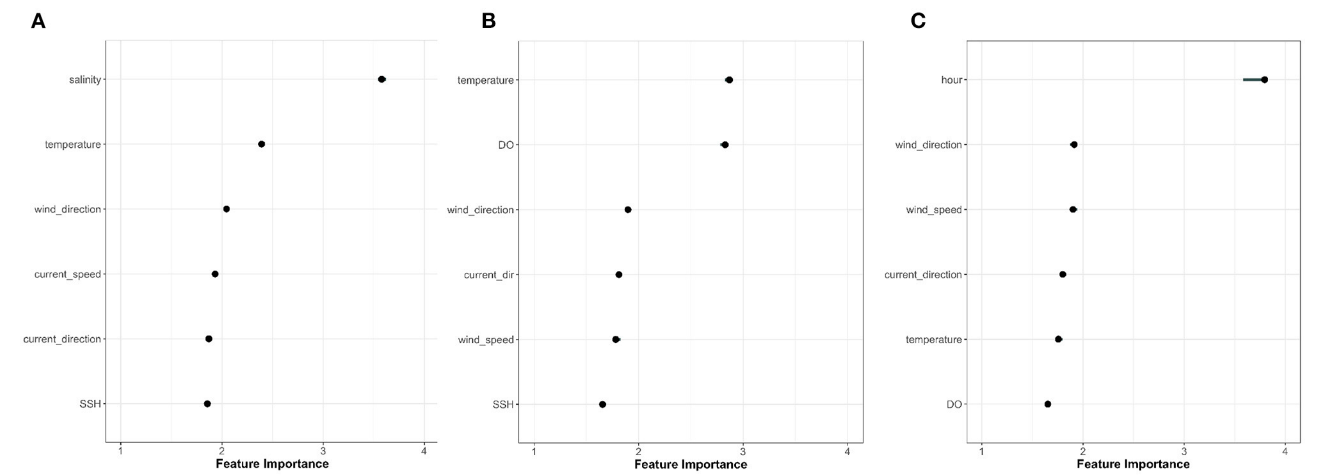

FIGURE 9 | Feature importance reported for our second experimental configuration "selected features" (section 2.3) at the **(A)** NOR, **(B)** SCO, and **(C)** CAN sites. The *y*-axis reports the ranked list of features that contributed the most to variation in fish depth measurements, while the *x*-axis presents relative magnitude of those contributions. Ranking predictors in this manner can quickly help sift through large datasets and understand data trends (Kuhn and Johnson, 2013).

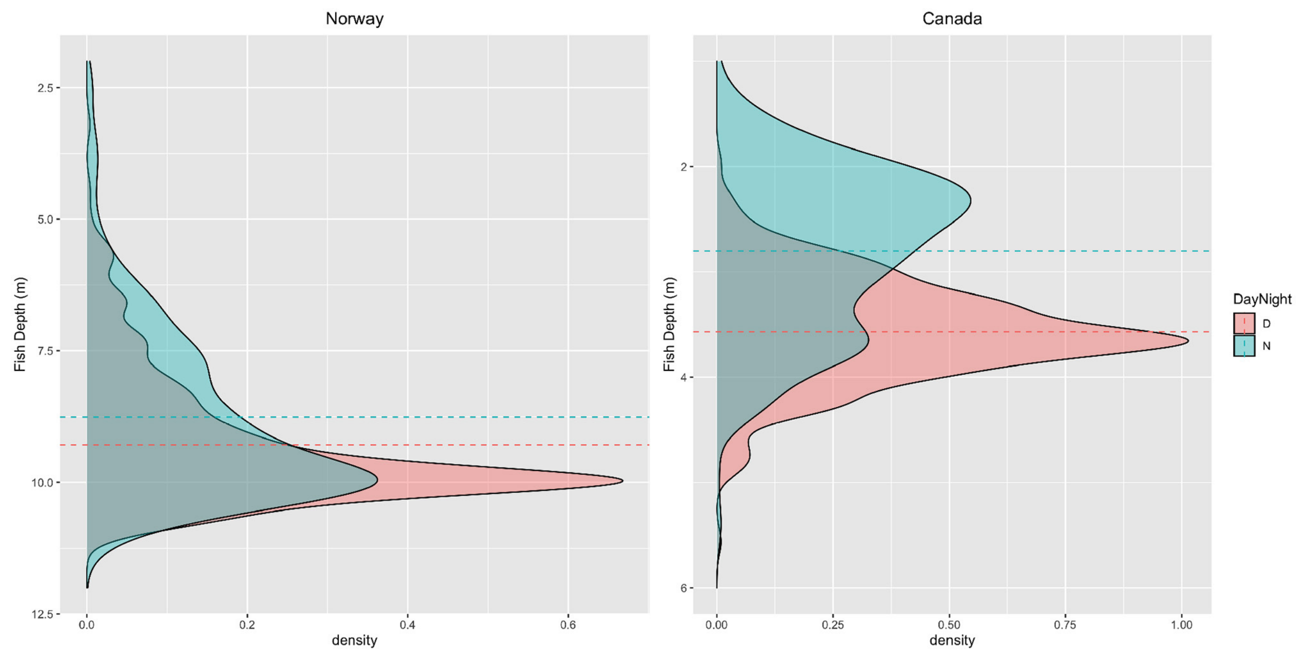

FIGURE 10 | Distribution of fish depth data for the NOR **(left)** and CAN **(right)** farm over the duration of the study period. The data is split into daytime and nighttime periods to explore how behaviours vary between those periods. The dashed vertical lines denote the mean for both periods.

Variation in oxygen levels were most pronounced at the CAN site which showed consistently lower values than at other locations (**Figure 5**). This is reflected in the feature importance analysis which denotes DO as an important contributor to fish position, and in particular indicates that lower values of oxygen have significant influence. **Figure 12** suggests that fish moved toward the surface when values drop below 7 mgL^{-1}. Values dropped below this threshold three times during the course of this study (**Figure 5**). This suggests that these low oxygen periods are worthy of additional study to understand how fish welfare were impacted and if additional behavioural modifications (e.g., horizontal swimming patterns or feeding activity) developed during these times. Research studies indicate that (at temperature of 16° C oxygen levels of 7mgL^{-1} lead to reduced appetites in full-feeding Atlantic salmon, while values of 6mgL^{-1} initiated acute anaerobic metabolism, and increased skin lesions (CREATE, 2008; Oppedal et al., 2011).

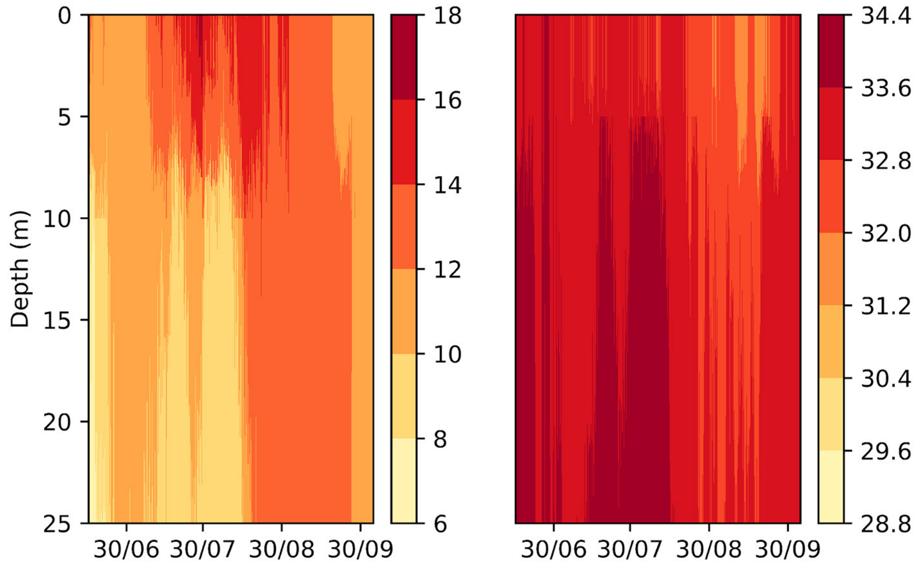

FIGURE 11 | Transect of temperature **(left)** and salinity **(right)** extracted from the Norkyst ocean model at the grid cell closest to the NOR farm. Colorbar denotes temperature in °*C* and salinity in units of PPU for the respective plots.

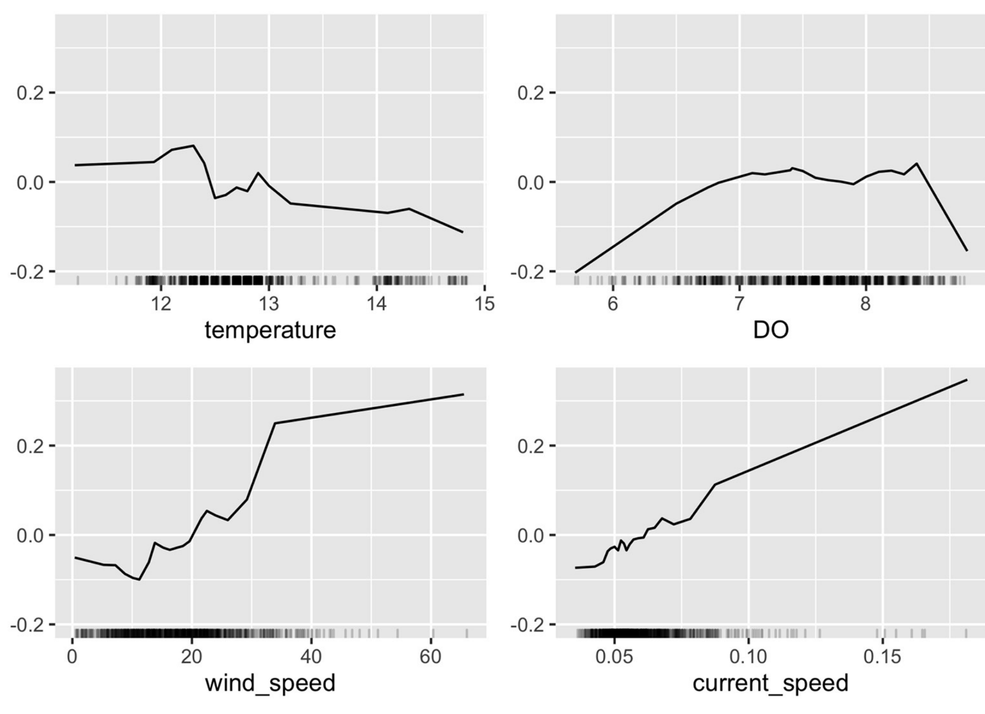

FIGURE 12 | Feature importance reported by the AutoAI model at the CAN site for a subset of environmental variables using *accumulated local effects* computation. ALE provides an efficient way to explore how much the target variable (fish depth) in response to selected feature (temperature, DO, wind speed, and current speed). The y-axis report the accumulated local effects (ALE) of each feature or variable in the units of the prediction variable (*m*), while the x-axis reports the range of values for each selected feature. Clockwise from top left results are presented for temperature, DO, current speed, and wind speed. ALE is a valuable technique to explore *how much* predictand varies in response to changes in predictors.

As alluded to in previous paragraphs, our approach only considered group behavioural responses in the vertical. Salmon typically form a circular swimming patterns that avoids both the innermost part and edges of the cage. These patterns breakdown at low stocking density, during feeding, at nighttime, or when threatened by a predator (Oppedal et al., 2011). Further, there are important interactions that happen at the individual level, such as aggression, that are not captured here. Aggression has been shown to vary as a function of stocking density and during feeding times (Adams et al., 2007), but it is not possible to resolve at the group level. While there is potential to leverage computer vision technology to monitor at the three-dimensional (Deakin et al., 2019), or individual level (Tidal, 2020), these are currently at a laboratory or research stage. An alternative approach is to tag individual fish to collect continuous data on the three-dimensional positioning of individual fish within a cage (Roy et al., 2014). These provide very high temporal resolution of position but only a small subset of the total fish in the cage can be feasibly tracked.

Approaches based on sampling at the individual level bear similarities to more established farm management practises in agriculture. Farmers have begun to use RFID systems to track livestock movement and health in order to improve health and welfare of terrestrial livestock. These systems have provided health information such as internal body temperature, growth performance, and even hold medical information; they have also provided movement data that provides information on behaviour and interaction between individuals (Ruiz-Garcia and Lunadei, 2011). Aquaculture faces similar challenges as livestock agriculture, and therefore lessons can be adapted and applied. However, additional challenges arise when animals can move in three-dimensions, and environmental conditions affect health and welfare more consistently than in agriculture. Fish are much more dependent on farmers for food, population density, and environmental conditions (Føre et al., 2018). Further, agriculture make wide use of radio frequency communication methodologies that are not feasible underwater and instead must rely on acoustic communication channels that are less technologically mature (Stojanovic and Preisig, 2009). One of the most striking differences between both industries is that livestock farming has been occurring for millennia, where salmon farming has only been active for the last few decades, and therefore new methods and technologies to understand animal health and welfare becomes a more challenging task and requires diligent research and cooperation between farmers and researchers.

In this paper, we explored statistical and machine learning approaches to explore environmental drivers of fish behaviour using environmental observations and hydroacoustic sensors. A major challenge in research such as this is the data collection step and this varies depending on the location. In CAN, farm sites tend to be relatively shallow, ranging between 10–15 m, and in remote coastal communities. Hydroacoustic systems are successful in collecting data on high density fish populations when cage depths allow for full view. In CAN, CageEye was unable to provide a full view of the cage due to the shallowness with only 11 m of depth available when the system was designed to be placed nearer to 15–20m (pers. comm). Without the ability to place a hydroacoustic system deeper in the water column, only a small view is available. Data quality issues can be exacerbated as a result of acoustic interference from other instruments within the cage and reflection from sea surface or site bottom. Furthermore, in order to study fish behaviour, a 24-h view is needed requiring consistent clean power to be run throughout the site. With farms in more remote locations, many sites use gas-powered generators which often shut down and require maintenance as well as constant observation. In order to successfully run hydroacoustic systems at shallow sites, a clean consistent power source that can provide energy for 24-h a day would allow for uninterrupted data collection.

Results presented in this paper indicate pronounced differences between sites and the need to consider these variations for farm management. One could readily use this approach to quantify the difference between sites, and further to identify the fundamental drivers to these variations. This could be particularly valuable when comparing different farm systems such as inshore and offshore and the associated operational implications.

The primary advantage of the hydroacoustic datasets presented here are the relative ease of collection of high-density measurements of fish behaviour. This paper presents a framework to identify the dominant environmental variables influencing fish behaviour (i.e., vertical motion), and extract insight on how changes in the environment affect fish response (e.g., **Figure 12**). On the other hand, these datasets only serve as a proxy for key performance metrics that might be collected on farms, such as feeding activity or satiation, fish health as measured by things such as gill status or lice count, and mortalities. In follow-on work we will explore whether welfare indices collected on farms can be explained or predicted by a combination of sensor datasets (hydroacoustic measurements and environmental observations). In particular, we will investigate how relatively high-density, population level measurements such as hydroacoustic data can inform more sparse individual-level measurements such as sea-lice, gill health, mortalities, etc.). Further, this study considered fish behaviour in terms of group vertical movement patterns. In subsequent work, we will deploy fish tags to monitor individual fish in three-dimensions to better encapsulate individual movement patterns in three dimensions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

FO'D, SP, and JG contributed to conception and design of the study. CS, GM, and CW collected and organised the data. FO'D wrote the first draft of the manuscript. CS, SP, GM, and RF wrote sections of the manuscript. FO'D and PP performed the statistical analysis. All authors contributed to manuscript revision, read, and approved the submitted version.

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