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Impact of betaine on laying hens' productive and physiological reactions at the late stage of production

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Introduction: Due to the anti-inflammatory, antioxidant, and antibacterial properties of betaine, it may serve as a beneficial feed supplement for laying hens. This research aimed to evaluate whether betaine enhances the productive, reproductive, and intestinal health of layer hens.

Methods: A total of 240 laying hens, aged 60 weeks, were divided into four treatment groups, each consisting of 60 hens. Each group was further divided into four replicates of 15 hens each. The first group, designated as the control (CTRL), received only the basal diet. The other treatment groups received the basal diet supplemented with betaine at levels of 0.3, 0.6, and 1.2 g/kg. The experiment was conducted over 24 weeks.

Results: The results indicated that the addition of betaine improved the growth performance traits of Fayoumi laying hens. Specifically, the inclusion of 0.6 g of betaine per kilogram of feed led to increased fertility ($p \leq 0.05$) and hatchability rates. In addition, betaine treatment significantly enhanced ($p \leq 0.05$) both the absolute and relative carcass weights while reducing abdominal fat. Moreover, a betaine level of 1.2 g/kg improved the lipid profile by lowering the serum levels of total lipids, cholesterol, and low-density lipoprotein (LDL). This level also significantly ($p \leq 0.05$) increased the counts of beneficial bacteria, including the total bacterial count (TBC) and Lactobacillus, in the cecum while reducing the count of pathogenic bacteria.

Discussion: Overall, dietary treatments with betaine, particularly at levels of 0.6 and 1.2 g/kg, could enhance the performance, carcass traits, blood indices, and cecal bacterial populations of Fayoumi laying hens.

KEYWORDS

Fayoumi layer hens, reproductive performance, blood indicators, carcass traits, intestinal microbiota

1 Introduction

Feed additives are essential for the poultry sector, providing a significant amount of animal protein. Feeding constitutes roughly 60%–70% of the expenses associated with poultry rearing. Therefore, effective feed usage is critical (Elsherbeni et al., 2024a; Elsherbeni et al., 2024b; Kamal et al., 2025; Saeed et al., 2025). In addition, antibiotics are no longer permitted as growth enhancers in poultry production due to their long-term use resulting in the emergence of bacterial resistance and the buildup of antibiotic residues in poultry products such as eggs and meat (Al-Khalaifa et al., 2019; Al-Khalaifah et al., 2020; Abd El-Hack et al., 2023). To improve the immunity and productivity of birds and farm animals, organic nutritional supplements have recently supplanted synthetic growth enhancers and antibiotics to augment the growth efficiency and enhance the carcass characteristics (Kamal et al., 2023; El-Ratel et al., 2024; Ali et al., 2025).

Betaine (Bet), a trimethylglycine derivative, is vital in the diet of birds and is used by plants and animals for the production of methionine, carnitine, and creatine (Arif et al., 2022; Elmahdy et al., 2025). Bet is associated with several biological processes, including immunology, fat distribution, methionine and choline sparing, and osmo-protection (Attia et al., 2019; Arumugam et al., 2021). It is a safe and effective feed additive commonly used in poultry as it promotes growth, reduces inflammation, and protects against oxidative stress (Shakeri et al., 2018). It is an important component of improving performance and stress tolerance (Yang et al., 2025). Bet is crucial for improving poultry performance by protecting the intestinal cell proteins and enzymes, preventing dehydration, promoting water retention, and lowering the body temperature in heat-stressed hens (Lan and Kim, 2018; Al-Qaisi et al., 2023). In addition, Liu et al (2019) found that the addition of Bet at levels of 0.50, 1.0, and 2.0 g/kg into broiler diet reduced the negative impact of heat stress on the feed intake and body weight. Bet has been shown to reduce fat; moreover, it has antioxidants, anti-coccidiosis, and anti-stress properties (Ahmed et al., 2018; Abd El-Ghany and Babazadeh, 2022). Moreover, it enhances the carcass yields and muscle protein deposition in broilers (Chen et al., 2022). The addition of Bet into the feed led to increased weight gain (WG), final body weight (FBW), and feed intake (FI) (Al-Sagan et al., 2021; De Prekel et al., 2024). According to Park and Kim (2019), the addition of Bet into diets with enough methyl group donors increased the feed efficiency and WG by approximately 3%–15%.

Dual-purpose hens (both meat and egg production) raised under prolonged heat stress and supplemented with 200 mg vitamin C and 1,000 mg Bet/kg diet showed improved egg-laying rates, albumen weight, yolk weight, and Haugh unit (HU), and this supplementation also helped birds tolerate heat stress (Attia et al., 2016). Hao et al (2017) reported that the health, laying performance, and egg production of layer chickens under heat stress improved after the addition of 400 mg Bet/kg diet. In quail, Ratriyanto et al (2017) suggested that the addition of Bet (at levels of 0.06%–0.12%) into quail layer diets increased the egg weight and egg yolk weight in birds raised in tropical environments. A previous study by Zaki et al (2023) found that Bet supplementation positively

impacted the immune response, egg quality assessment, and productive performance of Bovans Brown laying hens. Beneficial bacteria in poultry improve the health of host birds by competing with pathogenic bacteria and fostering the gut microbiota. Bet, despite its insufficient direct antibacterial impact, may prevent intestinal rupture, diarrhea, and dehydration; improve gut health; and enhance poultry resistance to *Clostridium* infections (Mohamed et al., 2024).

The growth-promoting and stress-relieving properties of Bet have been extensively studied, especially in broilers; however, its effects on laying hens—particularly during the later stages of production, when the physiological functions and egg quality regularly deteriorate—are not well understood. The late-laying stage is characterized by a reduced feed efficiency, a worse reproductive performance, and a compromised intestinal integrity. After 60 weeks of age, the laying rate and egg quality traits of laying hens—particularly the eggshell quality—decline (Molnár et al., 2016; Alfonso-Carrillo et al., 2021). Therefore, maintaining economic efficiency in egg production relies on identifying effective nutritional strategies to sustain the productivity and health at this stage. Due to the anti-inflammatory, antioxidant, and antibacterial properties of Bet, it might represent a useful feed supplement for old-age layers. Further research is needed to fully comprehend the influence of Bet on the late-laying egg production period and egg quality (Guo et al., 2023). Thus, our study hypothesizes that Bet can serve as an effective dietary additive, promoting growth and health outcomes. Thus, the goal of this research was to determine whether this natural additive can enhance the productive, reproductive, and intestinal health of old-age layer hens.

2 Materials and methods

2.1 Ethical approval

The present investigation was conducted on an Alnajem Poultry Farm, located at 28°36'57.3" N, 48°05'26.1" E, Al Wafra City, Kuwait, under the supervision of the Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, Kuwait. The Institutional Animal Care and Use Committee and the Kuwait Institute for Scientific Research Ethics Committee assessed and approved all experimental protocols at the Kuwait Institute for Scientific Research. The approvals ensured that all research activities complied with ethical standards for the treatment and utilization of animals in studies.

2.2 Birds and management

A total of 240 laying hens, aged 60 weeks, were divided into four treatment groups, each consisting of 60 hens. Each group was further divided into four replicates of 15 hens each in a completely random design. The average body weight and daily egg production of each group were approximately similar. The birds

were housed in identical controlled sanitary and climatic conditions and were provided unlimited access to a basal layer diet (Table 1), which was created based on the suggested requirements of the National Research Council (NRC) (National Research Council, 1994). The duration of daylight during the trial period was set to 16 h each day. All birds were housed in floor-laying shelters measuring 3 m × 2 m. The experiment was conducted from 60 to 84 weeks, encompassing 24 weeks of egg production.

2.3 Experimental design

The Bet (synthetic betaine hydrochloride) used in the current study was obtained from Shandong Ruihong Biotechnology Co., Ltd. (Dezhou, China) and contains ≥98% Bet. To guarantee homogeneity, Bet (in a powder form) was completely premixed with a small amount of the basal diet (in a mashed form) and then blended into the full feed batch for 30 min using a mechanical mixer. An experiment was conducted utilizing four different dietary Bet concentrations (0, 0.3, 0.6, and 1.2 g/kg diet). The first group

(control, CTRL) consumed only the basal diet. The remaining three experimental groups consumed the basal diet with the addition of Bet at a concentration of 0.3, 0.6, or 1.2 g/kg.

2.4 Productive performance

We weighed each hen to the nearest gram at the start of the trial and monthly thereafter. Furthermore, the diet of each experimental replicate was weighed daily, and then the average FI/hen was determined by collecting and weighing the leftover diet. The FI was recorded. Moreover, the feed conversion ratio (FCR) was calculated according to the following equation:

$$\text{FCR} = \text{average feed consumed(g)} / \text{average egg mass(g)}$$

2.5 Laying performance

For each replicate, the eggs were physically retrieved twice a day during the study period. The number of eggs (EN) and the weight of the eggs (EW) were both noted down to the nearest gram. To determine the egg mass (EM) for each replicate in each experimental group, we multiplied the total EN laid by the average EW during the experiment. We also used the following formula to calculate the monthly laying rate (LR) for each replicate:

$$\text{LR} = (\text{EN} / \text{living hen number}) \times 100$$

Monthly, a total number of 20 fresh eggs were taken from each replicate (80 eggs/treatment) over three consecutive days for incubation. Before incubation, each egg was labeled and kept at 18°C with 70% relative humidity. The eggs were automatically rotated every 2 h during incubation at the standard settings of 37.8°C and 55%–60% relative humidity. The eggs were candled on day 7 of incubation to detect infertile eggs, which were then noted and removed. At the end of the incubation period, the total number of hatched chicks was recorded. The methods outlined by Attia et al (2019) and El-Ratel et al (2024) were employed for the incubation process, as well as the fertility (in percent) and hatchability (in percent) calculations. The fertility and hatchability were calculated as follows:

$$\text{Fertility}(\%) = (\text{total number of fertile eggs} / \text{total eggs set}) \times 100$$

$$\text{Hatchability}(\%) = (\text{total number of hatched chicks} / \text{total number of fertile eggs}) \times 100$$

2.6 Egg quality traits

For each month of the experiment, eight fresh eggs per treatment were randomly chosen, weighed to the nearest gram, and separated. A digital caliper was used to measure the length, width, yolk height, diameter, and albumen height of the egg. The

TABLE 1 Composition and calculated analysis of the basal experimental diet.

Ingredient	Value
Yellow corn (8.5%)	61.17%
Soybean meal (44%)	16.70%
Wheat bran (15.7%)	6.70%
Corn gluten (60%)	4.70%
DL-Methionine (99%)	0.51%
Limestone (CaCO ₃)	8.13%
Dicalcium phosphate	1.42%
Salt (NaCl)	0.37%
Vitamin and mineral premix ^a	0.30%
Total	100%
Calculated analysis^b	
ME (kcal/kg)	2,699
Crude protein (%)	16.02
Crude fiber (%)	3.47
Calcium (%)	3.45
Available phosphorus (%)	0.40
Lysine (%)	0.73
Methionine (%)	0.34
Methionine + cysteine (%)	0.62
Na (%)	0.19

^aSupplied per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 2,200 IU; vitamin E, 10 mg; vitamin K3, 2 mg; vitamin B1, 1 mg; vitamin B2, 5 mg; B6, 1.5 mg; B12, 10 µg; nicotinic acid, 30 mg; folic acid, 1 mg; pantothenic acid, 10 mg; biotin, 1.5 µg; choline, 250 mg; copper, 10 mg; iron, 30 mg; selenium, 0.1 mg; cobalt, 0.1 mg; manganese, 60 mg; zinc, 50 mg; and iodine, 1 mg.

^bCalculated according to the National Research Council (NRC) (Molnár et al., 2016).

weights of the yolk and the shell were measured using a highly precise scale with a sensitivity of 0.01 g. The thickness of the shell, including the membranes, was measured using a micrometer to the nearest 0.01 mm. The egg shape index was determined by dividing the width of the egg by its length (Anderson et al., 2004). In addition, the yolk index was calculated by division of the yolk height by the diameter multiplied by 100 (Kondaiah et al., 1983). The egg HU was determined using the formula proposed by Moula et al (Moula et al., 2013).

2.7 Carcass and blood biochemical parameters

At the end of the experiment (84 weeks), two chickens from each treatment group were randomly selected to measure the carcass traits. Hens were weighed separately, kept without food overnight (but with free access to water), and then slaughtered by hand to determine how much the carcass and edible components weighed. Blood samples were taken from two hens per replication (the same birds used to examine the carcass traits) and were placed into tubes that did not have heparin. Subsequently, at 4°C and 5,000 rpm, the samples were spun in a centrifuge to obtain the serum. This was maintained at −20°C until analysis. The serum was assessed utilizing commercially available assays. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured, along with the total protein (TP), albumin (Alb), globulin (gl), total lipid (TL), cholesterol (Cho), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels.

2.8 Microbiological analysis

After carcass evaluation, 10 g of each bird's cecum was collected, mixed with sterilized peptone water (90 ml), and then stirred for 30 min. The supernatant was taken, and serial dilutions up to 10^{-7} were set, as suggested by Abd El-Hack et al (Abd El-Hack et al., 2025). "The TBC, *Lactobacilli*, *E. coli*, coliforms, and *Salmonella* spp." were counted on the growth media, as described by Abd El-Hack et al (Anderson et al., 2004). Bacterial counts were transformed to logarithm of colony-forming units per gram (log CFU/g) (Sheiha et al., 2020).

2.9 Statistical analysis

Statistical data analysis in the SAS application utilized the general linear model (GLM) technique (SAS Institute, 2004). Duncan's multiple range test was used to assess distinct differences among the means of several treatments (Duncan, 1995). The study employed the following statistical model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where Y_{ij} is the value of the corresponding variable, μ is the overall mean of the corresponding variable, and T_i is the effect caused by the i th Bet concentration, where $i = 1, 2, 3$, and 4 ($1 = 0, 2 = 0.3, 3 = 0.6$, and $4 = 1.2$ g Bet/kg diet). ε_{ij} is a random error related to the ij th observation and is presumed to be independently and normally distributed. Significant difference was considered at $p \leq 0.05$.

3 Results

3.1 Growth efficiency

Table 2 illustrates the impact of Bet supplementation on the live body weight (LBW), FI, and FCR of Fayoumi hens throughout the experimental period. There was a significant ($p \leq 0.05$) influence of Bet levels on the FBW and FI only. Bet addition increased the layers' FBW, especially at the level of 0.6 g/kg diet, followed by 0.3 and 1.2 g/kg diets. Moreover, the FI decreased gradually with increasing Bet levels. Noticeably, the lowest FI values were obtained from layers fed 1.2, 0.6, and 0.3 g Bet/kg diet. Although the FCR was not significantly affected by Bet treatment, the lowest FCRs were obtained from layers fed 0.6 g Bet/kg diet, followed by 1.2 and 0.3 g Bet/kg diets, in comparison to the CTRL group. According to these findings, enhanced growth performance was up to the level of 0.6 g/kg, after which the impact plateaued.

3.2 Laying production

The data illustrated in Table 3 display the impact of Bet on the egg production and reproduction parameters. It was apparent that dietary supplementation with Bet meaningfully ($p \leq 0.05$) improved the LR, EM, and fertility (in percent), with an insignificant increase in EW and hatchability (in percent). Layers receiving 0.6 g Bet/kg

TABLE 2 Growth performance of Fayoumi laying hens as affected by the addition of different levels of betaine (Bet) (mean $\pm$ SE).

Trait	Betaine (g/kg diet)				p-value
	0.0	0.3	0.6	1.2	
Initial BW	1,453.38 $\pm$ 7.15	1,455.61 $\pm$ 5.38	1,455.17 $\pm$ 6.07	1,453.87 $\pm$ 4.75	0.984
FBW	1,961.83 $\pm$ 12.01bc	1,981.31 $\pm$ 9.57b	2,024.37 $\pm$ 7.35a	1,953.86 $\pm$ 7.35c	0.001
FI	107.41 $\pm$ 1.34a	104.34 $\pm$ 1.29ab	102.59 $\pm$ 1.28b	102.30 $\pm$ 1.22b	0.022
FCR	3.13 $\pm$ 0.35	3.00 $\pm$ 0.36	2.91 $\pm$ 0.19	2.96 $\pm$ 0.15	0.734

Means within a column with different lowercase letters are significantly different ($p \leq 0.05$). BW, body weight; FBW, final body weight; FI, feed intake; FCR, feed conversion ratio.

diet achieved the highest rates of these parameters compared with those on other Bet levels and those in the CTRL group. The addition of Bet had no noticeable impact on hatchability or EW. This suggests that, for productive and reproductive efficiency, a modest supplementation level (0.6 g/kg) is ideal.

3.3 Egg quality

Table 4 shows the influence of Bet addition at different levels into Fayoumi layers' diet on their egg quality characteristics. There was a substantial increase ($p \leq 0.05$) in each egg yolk index and eggshell thickness due to Bet treatment. Compared with the different treatments and the CTRL group, Bet at 0.6 g/kg diet exhibited the highest values for all egg quality traits. The other egg quality traits (e.g., albumen index, shape index, and HU) were not significantly affected by the Bet levels.

3.4 Carcass traits

The impact of the different Bet levels on the carcass traits of Fayoumi layers is summarized in Table 5. All carcass traits were affected ($p \leq 0.05$) by the addition of Bet, except for the absolute and relative weights of giblets. The addition of 0.6 g Bet/kg diet improved all carcass traits when compared with the other Bet

levels and the untreated group. Moreover, both the absolute and relative abdominal fat weights were meaningfully ($p \leq 0.05$) diminished due to the addition of different levels of Bet, especially at 0.6 g.

3.5 Blood parameters

The blood biochemical indices, as well as the antioxidant enzyme activity, represent the general health and nutritional status of poultry. The data presented in Table 6 indicate the effects of Bet treatment on the different blood indices. Notably, Bet significantly ($p \leq 0.05$) affected the LDL and AST levels only. Compared with the other levels of Bet added, 1.2 g/kg exhibited the lowest levels of TLLs, Cho, and LDL, while the highest level of HDL was obtained from the addition of 0.3 g/kg Bet. Regarding the liver enzymes, the addition of 0.6 and 1.2 g/kg Bet showed the highest levels of AST and ALT, respectively. The highest values of TP, Alb, gl, as well as the albumin-to-globulin (A/G) ratio, were obtained from the addition of 0.3 g/kg Bet compared with other treatments.

3.6 Microbiological analysis

The types of bacterial cultures and specific phytochemicals in the intestines of poultry were found to be significantly correlated,

TABLE 3 Egg production and reproduction parameters of Fayoumi laying hens affected by the addition of different levels of betaine (Bet) (mean $\pm$ SE).

Trait	Betaine (g/kg diet)				<i>p</i> -value
	0.0	0.3	0.6	1.2	
Laying rate (%)	64.27 $\pm$ 0.60b	64.47 $\pm$ 0.37b	66.93 $\pm$ 0.57a	65.52 $\pm$ 0.64ab	0.014
Egg weight (g)	48.39 $\pm$ 0.39	48.71 $\pm$ 0.57	49.61 $\pm$ 0.53	49.41 $\pm$ 0.55	0.490
Egg mass (g/hen)	32.81 $\pm$ 0.28b	33.32 $\pm$ 0.30ab	34.02 $\pm$ 0.26a	33.11 $\pm$ 0.22b	0.011
Fertility (%)	90.28 $\pm$ 0.71b	90.70 $\pm$ 0.46ab	91.76 $\pm$ 0.31a	91.16 $\pm$ 0.32ab	0.040
Hatchability (%)	86.17 $\pm$ 0.54	86.37 $\pm$ 1.23	87.38 $\pm$ 0.57	86.38 $\pm$ 0.46	0.420

Means within a column with different lowercase letters are significantly different ($p \leq 0.05$).

TABLE 4 Egg quality traits of Fayoumi laying hens affected by the addition of different levels of betaine (Bet) (mean $\pm$ SE).

Trait	Betaine (g/kg diet)				<i>p</i> -value
	0.0	0.3	0.6	1.2	
Shape index (%)	73.17 $\pm$ 1.37	75.66 $\pm$ 0.30	75.98 $\pm$ 0.92	74.53 $\pm$ 0.62	0.117
Yolk index (%)	35.80 $\pm$ 3.57b	37.40 $\pm$ 2.59a	38.00 $\pm$ 5.29a	37.40 $\pm$ 6.40a	0.015
Albumen index (%)	86.08 $\pm$ 1.96	79.43 $\pm$ 1.81	86.60 $\pm$ 3.26	86.52 $\pm$ 2.41	0.820
HU	76.54 $\pm$ 1.58	79.85 $\pm$ 1.50	83.38 $\pm$ 2.63	79.96 $\pm$ 1.15	0.375
Shell thickness (mm)	0.30 $\pm$ 0.04b	0.32 $\pm$ 2.88a	0.33 $\pm$ 3.90a	0.32 $\pm$ 4.20a	0.001

Means within a column with different lowercase letters are significantly different ($p \leq 0.05$).
HU, Haugh unit.

TABLE 5 Carcass traits of Fayoumi laying hens affected by the addition of different levels of betaine (Bet) (mean $\pm$ SE).

Trait	Betaine (g/kg diet)				p-value
	0.0	0.3	0.6	1.2	
Pre-slaughter weight (g)	1,887.83 $\pm$ 107.55	1,897.92 $\pm$ 70.34	1,937.67 $\pm$ 64.67	1,950.75 $\pm$ 62.21	0.117
Carcass (g)	1,437.08 $\pm$ 103.43b	1,502.17 $\pm$ 91.16ab	1,617.50 $\pm$ 73.72a	1,494.58 $\pm$ 77.54b	0.032
Carcass (%)	68.97 $\pm$ 2.49ab	65.91 $\pm$ 2.24ab	70.75 $\pm$ 1.90a	65.59 $\pm$ 1.56b	0.050
Giblets (g)	83.41 $\pm$ 1.51	84.75 $\pm$ 2.03	87.41 $\pm$ 2.55	85.25 $\pm$ 2.31	0.650
Giblets (%)	4.11 $\pm$ 0.18	3.84 $\pm$ 0.15	4.02 $\pm$ 0.18	3.97 $\pm$ 0.14	0.555
Edible meat (g)	1,520.50 $\pm$ 104.16b	1,586.92 $\pm$ 91.45ab	1,704.92 $\pm$ 73.40a	1,579.83 $\pm$ 77.93b	0.035
Edible meat (%)	72.91 $\pm$ 2.57ab	70.74 $\pm$ 2.17ab	77.25 $\pm$ 1.85a	72.68 $\pm$ 2.10b	0.033
Abdominal fat (g)	167.41 $\pm$ 4.61a	141.75 $\pm$ 5.74a	135.58 $\pm$ 7.62b	161.91 $\pm$ 3.16a	0.016
Abdominal fat (%)	8.34 $\pm$ 0.57a	6.36 $\pm$ 0.33a	6.15 $\pm$ 0.45b	7.48 $\pm$ 0.25a	0.060

Means within a column with different lowercase letters are significantly different ($p \leq 0.05$).

resulting in improved productive performance. The results presented in Figure 1 indicate a significant ($p \leq 0.05$) modification of the total bacterial count (TBC) and cecal microflora due to dietary supplementation with different levels of Bet. From the outcomes obtained, it is evident that Bet addition at levels of 0.6 and 1.2 g/kg produced the maximum TBC and *Lactobacillus* counts. Similarly, the same levels exhibited the lowest count of pathogenic bacteria (coliform, *Escherichia coli*, *Salmonella*, and *Clostridium*) compared with the other Bet levels and the CTRL group.

4 Discussion

Phytogenic additives have continuously shown success in improving a range of poultry performance traits in recent years (Al-Ardhi et al., 2020; Almrsmi et al., 2021). Bet is a safe and effective feed supplement that promotes growth and lipotropic activity. As a result, it is widely utilized as a nutritional supplement for many poultry species. Furthermore, it has antioxidant and anti-inflammatory properties. According to the results obtained in the current trial, the addition of different levels of

TABLE 6 Blood parameters of Fayoumi laying hens affected by the addition of different levels of betaine (Bet) (mean $\pm$ SE).

Trait	Betaine (g/kg diet)				p-value
	0.0	0.3	0.6	1.2	
Lipid profile					
TLs (mg/dl)	101.28 ± 18.89	123.26 ± 18.89	164.53 ± 18.89	64.14 ± 18.89	0.900
Cho (mg/dl)	106.94 ± 8.23	123.15 ± 8.23	109.54 ± 8.23	107.27 ± 8.23	0.230
HDL (mg/dl)	11.69 ± 3.26	13.83 ± 3.26	13.00 ± 3.26	11.90 ± 3.26	0.510
LDL (mg/dl)	0.05 ± 0.02bc	0.09 ± 0.02ab	0.10 ± 0.02a	0.06 ± 0.02ab	0.008
Liver enzyme					
AST (mg/dl)	0.77 ± 0.07ab	0.73 ± 0.09b	0.81 ± 0.02a	0.79 ± 0.03a	0.004
ALT (mg/dl)	0.55 ± 0.05	0.56 ± 0.08	0.61 ± 0.08	0.64 ± 0.06	0.740
Protein fraction					
TP (mg/dl)	4.58 ± 0.33	4.94 ± 0.33	4.84 ± 0.33	4.27 ± 0.33	0.660
Alb (mg/dl)	2.20 ± 0.12	2.80 ± 0.12	2.25 ± 0.12	1.97 ± 0.12	0.730
gl (mg/dl)	2.67 ± 0.21	3.04 ± 0.21	2.79 ± 0.21	2.56 ± 0.21	1.230
A/G ratio	82.39 ± 1.26	92.10 ± 1.26	80.64 ± 1.26	76.95 ± 1.26	0.540

Means within a column with different lowercase letters are significantly different ($p \leq 0.05$).

TLs, total lipids; Cho, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TP, total protein; Alb, albumin; gl, globulin.

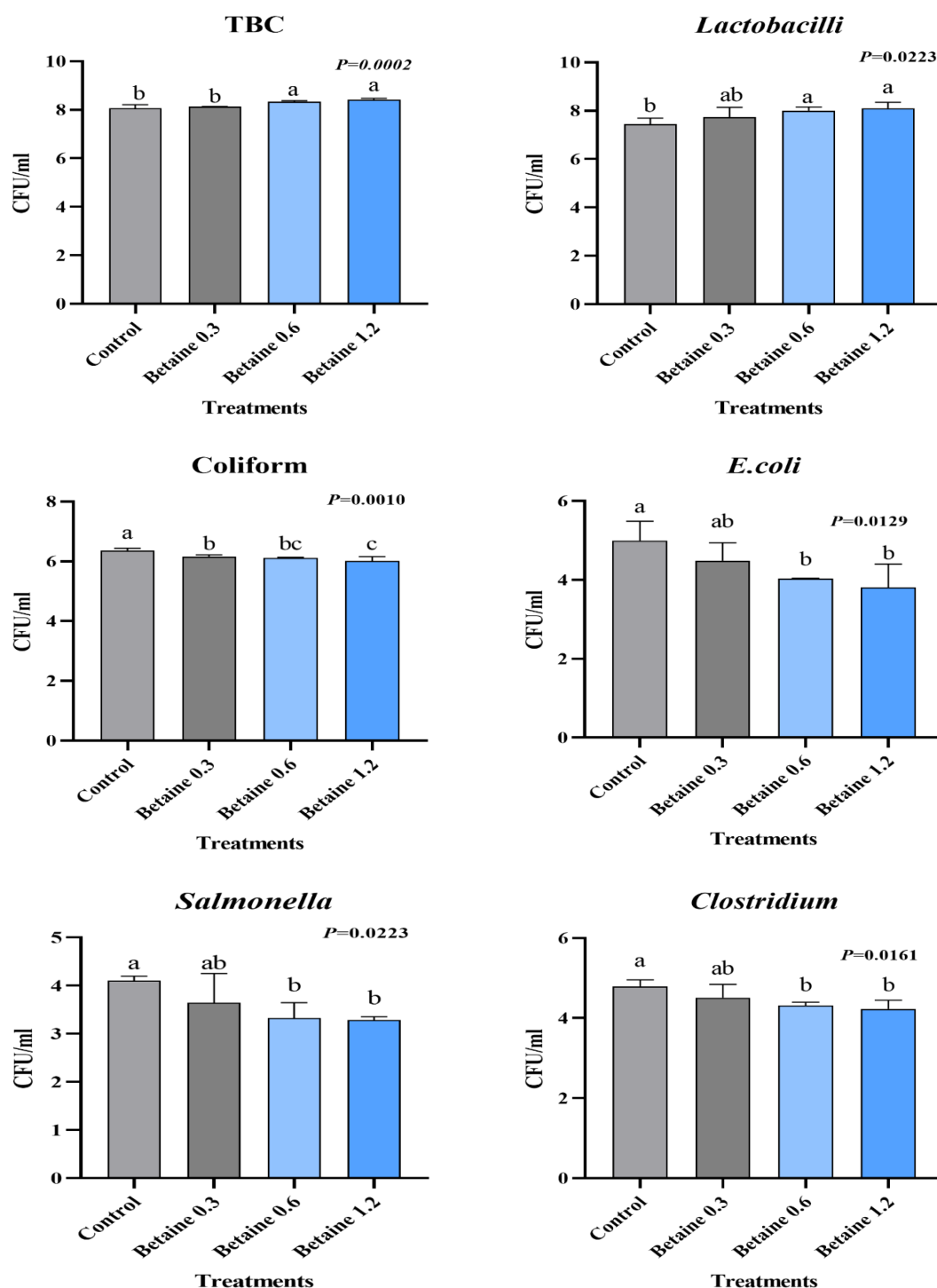


FIGURE 1

Cecal microbial counts [in colony-forming units (CFU) per milliliter] in Fayoumi laying hens affected by the addition of different levels of betaine (Bet). a, b, and c above the columns, with different superscripts, are significantly different ($P \leq 0.05$).

Bet improved the FBW, FI, and FCR. Our results are in the same line as those of Rao et al (Rao et al., 2011), who found that supplementing broiler diet with 1.0, 1.5, and 2.0 g Bet/kg resulted in significant increases in FI and LBW, as well as an improvement in FCR. Similarly, several researchers indicated that Bet improves the

growth performance, FCR, and carcass breast yield (Dos Santos et al., 2019).

Furthermore, Savaram et al (2021) and Elmahdy et al (2025) found that the body weight gain (BWG) and FI were meaningfully augmented when broilers were fed diets with different levels of Bet

in comparison to broilers fed a free-methionine basal diet. Furthermore, both the body weight and BWG, as well as the FI, increased when 25-week-old Lohmann Brown laying hens were fed diets containing 0.35% methionine and 0.75 g Bet/kg feed for 15 weeks (Omara et al., 2023). Bet supplementation may improve the digestibility of crude fiber, dry matter, and protein and promote mucosal expansion in the intestines, resulting in improved growth performance (El-Husseiny et al., 2007; Gonzalez-Rivas et al., 2020). Bet supplementation in birds enhances nutrient utilization, leading to the greater availability of sulfur-containing amino acids such as methionine and cysteine for muscle protein deposition and reducing the requirement for metabolizable energy (Awad et al., 2022). Conversely, Park and Ryu (2011) noted that the FI and FCR were not significantly affected when laying hens were fed a diet with 0.60 g/kg Bet.

In this work, it was revealed that there is a greater need for research on the impacts of Bet on the egg production, egg quality, fertility, and hatchability of laying hen strains. Although Bet addition did not significantly affect the EW and hatchability (in percent), it improved all of the egg production and reproduction traits, particularly when added at 0.6 g. A similar study by Guo et al (2023) and Xing and Jiang (2012) found that supplementing laying hen diets with Bet increased the egg production percentage. Gudev et al (2011) also noted that the egg production rate was significantly improved in laying hens fed diets added with 0.7 and 1.5 g Bet/kg, with an insignificant enhancement in the EW and EM. In addition, incorporating Bet into the diet at a dose of 1 g/kg enhanced the FI, oviduct index, LR, and EM in laying hens (Attia et al., 2016; Hao et al., 2017). Bet appears to reduce the requirement for additional CH₃ donors, such as choline and methionine, while enhancing the ability of the liver to synthesize proteins and fatty acids, ultimately resulting in improved egg production rates (Zaki et al., 2023). Bet enhances the egg production rates likely by promoting ovarian health and improving the key antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT), thereby exhibiting antioxidant properties in the intestinal tract and ovary of older laying hens (Du et al., 2025).

In the current study, as the dietary Bet levels increased up to 0.6 g/kg diet, there was a noticeable improvement in both egg production and reproductive performance; thereafter, the response either plateaued or slightly declined. This indicates that, to maximize reproductive efficiency, the ideal level for Fayoumi laying hens at the late phase of production is 0.6 g/kg. The following decline after this point could be caused by an osmotic imbalance at greater Bet concentrations or the saturation of CH₃ group consumption. Similar dose-dependent effects were observed by Gudev et al (2011) and Guo et al (Guo et al., 2023), suggesting that while excessive amounts of Bet may not provide further benefits, moderate levels promote the physiological processes supporting reproduction and egg formation. The biological function of Bet as a CH₃ donor supporting hormone production, follicular development, and embryo viability is highlighted by the observed improvements in fertility (in percent) and hatchability (in percent) at 0.6 g/kg.

In this study, increasing the dietary Bet levels to 0.6 g/kg significantly enhanced the egg production and reproductive performance of Fayoumi laying hens. However, beyond this level, these improvements plateaued or slightly decreased, suggesting that 0.6 g/kg is optimal for maximizing the reproductive efficiency in the late production phase. The decline past this point may have resulted from osmotic imbalance or CH₃ group consumption saturation. The similar dose-dependent effects noted by Gudev et al (2011) suggest that while excessive Bet supplementation may be unproductive, moderate levels enhance the reproductive processes and egg formation. Bet serves as a CH₃ donor, aiding in hormone production, follicular development, and embryo viability, which was reflected in the improved fertility and hatchability at 0.6 g/kg (Guo et al., 2023). In addition, the administration of Bet improved the fertility and hatchability of Fayoumi laying hens in comparison to the CTRL group. A prior investigation demonstrated that injecting doses of Bet (5, 10, and 15 mg) into fertile incubated eggs from meat breeder hens significantly improved hatchability, likely due to its role in methylation processes essential for gluconeogenesis during hatching (Tanimowo et al., 2024). However, high doses of Bet (0.10 g/egg) administered *in ovo* were found to significantly reduce hatchability (Kadam et al., 2013).

Gholami et al (2015) found no significant effects of Bet at levels of 0.250 and 0.50 mg/egg on hatchability. However, improvements in the fertility rates may be linked to hormones such as lutein, triiodothyronine, estradiol, thyroxine, follicle-stimulating hormone, and progesterone, which increase with dietary Bet (Zou XiaoTing et al., 1998). Specifically, Bet addition at 0.6 g improved the egg quality traits of Fayoumi hens, notably the egg yolk index and the eggshell thickness (Attia et al., 2016). Ryu et al (2002) also reported that 0.2% Bet in the diet can enhance the egg production and eggshell quality under heat stress conditions. In addition, in quails, the egg yolk and eggshell weights were meaningfully improved, while the Alb weight decreased due to the addition of 0.6 and 1.2 g Bet/kg, but did not significantly enhance the shell thickness (Abobaker et al., 2017; Ratriyanto et al., 2017). Adding 0.5% Bet to layer hens' diet at 65 weeks of production significantly increased the eggshell thickness. The thickness varies by breed, laying season, and diet, with Bet affecting the initial phase of eggshell formation rather than the final stage (Molnár et al., 2016). The EW, yolk color, and Alb width increased with the addition of Bet (0.125, 0.250, and 0.500 g/kg) in the diet of 57-week-old laying hens, with the highest values observed at 0.500 g. Bet also elevated the serum estrogen and melatonin levels, contributing to increased EM and production (Zaki et al., 2023).

The results from our study are in contrast to those of Gudev et al (Gudev et al., 2011), who reported no significant changes in the shell weight, egg Alb weight, egg yolk weight, and the HU scores with 0.7 and 1.5 g Bet/kg diet, although the shell thickness increased. Furthermore, the yolk%, albumen%, and eggshell% exhibited no significant alterations due to Bet at concentrations of 0, 0.70, 1.40, and 2.10 g/kg diet (Ratriyanto et al., 2018). The eggshell thickness significantly decreased with the addition of Bet at 1.5 g/kg in low-methionine diets. There were no significant

differences in the egg Alb weight, yolk weight, or HU among the various levels of Bet added. In addition, the shell quality declined due to the increase in EW, meaning that a constant shell is distributed across a larger egg (Omara et al., 2023).

The investigation indicates that the addition of varying doses of Bet, particularly at 0.6 g, enhances numerous carcass traits in Fayoumi layer hens. These results align with previous research showing increases in the carcass weight, the dressing percentage, and the giblet, thigh, and breast weights, alongside a significant reduction in the abdominal fat weight (Saeed et al., 2017; Abd El-Ghany and Babazadeh, 2022). There were clear differences in the weights of the different parts of broiler meat (i.e., drumstick, breast, shank, head, gizzard, liver, spleen, heart, and intestines) when different amounts of Bet were added into the diet. This may be related to the osmotic role of Bet in promoting water retention (Yeasmin T Jaman et al., 2023; Elmahdy et al., 2025). Moreover, the improvements in the carcass traits could be attributed to the function of Bet as a methyl group donor, enhancing the availability of essential amino acids (methionine and cysteine) crucial for protein synthesis (McDevitt et al., 2000; Neto et al., 2000). Previous studies have indicated that the effects of Bet supplementation on the carcass traits may be dependent on the methodology, animal species, and stress conditions (Sakomura et al., 2013; Yang et al., 2022; Kirrella et al., 2023).

In this study, the inclusion of 1.2 g Bet into the diet of Fayoumi laying hens improved their lipid profile by lowering the serum levels of Tls, Cho, and LDL, while all Bet doses increased HDL compared with the CTRL group. Similar findings were observed in broilers, where natural feed additives, including Bet, led to reduced blood lipid profiles and abdominal fat (Gholami et al., 2015; Xie et al., 2024). The role of the liver in converting fatty acids into triacylglycerols for transportation as very low-density lipoprotein (VLDL) was highlighted, and previous research confirmed that Bet decreased the triglyceride (TG) levels in both broilers and laying hens (Aljumaily et al., 2019; Hu et al., 2020; Zhao et al., 2022). Bet can induce glucocorticoid receptor (GR) gene production in poultry, which is involved in regulating processes of TG homeostasis and lipid metabolism (Wang et al., 2012; Omer et al., 2018). In addition, Bet prevents the accumulation of hepatic fat and boosts the mitochondrial activity and content. This evidence implies that Bet regulates fat metabolism (Zhang et al., 2019). The increased egg quality and TP and gl levels, along with the decreased Cho and LDL, may lead to an improved blood profile.

Wu et al (2024) demonstrated that Bet administration reduced the hepatic cholesterol buildup in corticosterone-induced laying hens by increasing the CYP7A1 and decreasing the HMGCR expression. In addition, the effect of Bet on lowering the blood lipids may have stemmed from the reduced hormone-sensitive lipase activity and the increased concentration of the methyl groups, promoting the formation of methylated compounds that enhance fat transport in the body, underscoring the importance of Bet in lipid metabolism (Zhan et al., 2006; He et al., 2015). Bet impacts the expression of the genes related to lipid transport,

affecting TG regulation and VLDL secretion (VerHague et al., 2013; Guo et al., 2020). In Cobb 500 broilers, the addition of Bet at 0.03%–0.12% into the drinking water increased HDL and decreased LDL and Cho, likely due to its antioxidant properties (Yeasmin T Jaman et al., 2023). Conversely, some studies reported higher Cho levels in broilers given 0.8 g/kg Bet without alterations in the TG levels (Rao et al., 2011).

The TP content in serum is crucial for the assessment of bird immunity, performance, and organ function. A study by Park and Kim (2019) identified that broilers on 1.2 g Bet/kg diet had higher total serum Alb levels. This decrease was attributed to the ability of Bet to contribute CH₃ in protein metabolism and to reduce protein loss by enhancing the intestinal integrity. Furthermore, Bet at 0.1 and 0.2%/kg diet diminished the ALT levels in broiler serum (Konca et al., 2008). Similarly, Mohamed et al (2022) found that the addition of 1 ml/L Bet into the drinking water lowered the Alb, gl, and TP levels while increasing ALT and AST. In contrast, Awad et al (2014) reported that incorporating 0.1 and 1.5 g Bet/kg in the diet of ducklings significantly lowered the ALT and AST activity. In contrast, adding Bet at levels of 0.125, 0.250, and 0.500 g/kg into the diet of laying hens at 57 weeks did not affect the plasma levels of AST, ALT, TP, Cho, gl, LDL, HDL, and TGs (Zaki et al., 2023; Elmahdy et al., 2025).

Poultry gut function is influenced by region, age, and production status. The small intestine is responsible for nutrient digestion and absorption, while the intestinal mucosal barrier protects against microbial threats (Peterson and Artis, 2014). The gut microbiota is essential for preserving intestinal health and enhancing immune function (Qi et al., 2021). In addition, when ducks underwent heat stress and were supplemented with 0.07%–0.13% Bet, they demonstrated an increase in microbial fermentation products and short-chain fatty acids (SCFAs) (Park and Kim, 2017). Bet exhibits antibacterial properties and enhances the antioxidant capacity, protecting gut cells from oxidative stress and inflammation, suggesting that it may also shield the intestinal microbiota from osmotic stress (Ratriyanto et al., 2009; Blagodatskikh et al., 2018; Wang et al., 2021). Our findings corroborate those of Pradista et al (Pradista et al., 2022), indicating that Bet, used as a feed additive at 0.12%/kg for ducks under heat stress, when supplemented at 0.07%–0.13%, resulted in an increased production of Clostridiaceae UCG-002 while decreasing *Olsenella*. Furthermore, Bet treatment significantly lowered the *Clostridium perfringens* counts *in vivo*, potentially due to improved cellular immunity characterized by leukocytosis, monocytosis, increased lymphocytosis, and enhanced phagocytic activity (Mohamed et al., 2022). Supplementing Lohmann laying pullets with Bet at 1.2 g/kg diet significantly increased the levels of various *Lactobacillus* species, as shown by Al Wahid et al (Al Wahid et al., 2024). This enhancement in beneficial bacteria may be attributed to the osmoprotective property of Bet, which promotes cell–bacteria concordance in the intestinal tract, and its ability to enhance the microbial fermentation of dietary fibers, resulting in improved fiber digestibility and increased production of SCFAs.

5 Conclusions

The addition of Bet, especially at the level of 0.6 g/kg diet, improved the growth performance, egg production, and quality. Moreover, it enhanced the fertility and hatchability rates and the blood indices in such a late period of production. The addition of high levels of Bet improved the cecal microbiota by increasing the beneficial bacteria and decreasing the pathogenic species. Henceforward, safe, healthy, and economical egg production could be achieved by utilizing Bet as a diet additive to layer hens at the late periods of production.

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 approved by The Institutional Animal Care and Use Committee and the Kuwait Institute for Scientific Research Ethics Committee assessed and approved all experimental protocols at the Kuwait Institute for Scientific Research. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

HA-k: Investigation, Visualization, Funding acquisition, Validation, Supervision, Project administration, Methodology, Conceptualization, Writing – review & editing. SA-A: Writing – original draft, Formal Analysis, Validation. HN: Validation, Formal Analysis, Writing – original draft. AA-N: Writing – original draft, Visualization, Resources, Data curation, Methodology, Conceptualization, Software. HK: Writing – original draft, Investigation, Visualization, Writing – review & editing. MS: Resources, Investigation, Writing – original draft. HA: Investigation, Data curation, Writing – original draft, Visualization.

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