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Effect of betaine hydrochloride on nutrient metabolizability and egg production parameters in layers

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Abstract
The present study was conducted on 160 layer birds to assess the effect of betaine hydrochloride supplementation on nutrient metabolizability and different egg production parameters in layers. The birds were randomly divided into five treatment groups namely B0.0, B0.5, B1.0, B1.5 and B2.0 and each group had 4 replicates of 8 birds in each. Group B0.0 served as the control and while B0.5, B1.0, B1.5 and B2.0 received 0.50, 1.00, 1.50 and 2.00 g betaine hydrochloride/kg feed, respectively. The percent mean values of dry matter metabolizability, gross energy metabolizabilty and nitrogen retention were all significantly higher due to betaine feeding in comparison to control diet and the highest nutrient metabolizabilty was observed in B2.0 group. The egg production indices like egg weight, hen day egg production and egg mass production were all significantly increased in betaine fed groups in comparison to control, with the highest value recorded in group B2.0 supplemented with betaine @ 2g/kg feed followed by B1.5, B1.0, B0.5 and B0.0. This study found that 2g betaine per kg feed was most advantageous in increasing nutrient metabolizability and egg production parameters among all graded levels of betaine when compared to the control diet.

Keywords: Betaine, nutrient metabolizability, hen day egg production, egg mass production

Introduction
Betaine, a naturally occurring trimethyl derivative of glycine, has emerged as a valuable feed additive in poultry nutrition due to its multifaceted benefits. This compound, found in various plant and animal products, plays a dual role in the body: as a methyl group donor and an organic osmolyte. As an organic osmolyte, betaine safeguards cells against osmotic inactivation, maintaining cell volume under heat stress conditions [1]. Additionally, betaine donates its methyl group to homocysteine, converting it into methionine. This methionine-sparing effect enables efficient utilization of dietary protein, positively correlated with body tissue growth [2]. Beyond its dual function, betaine has been found to modulate various growth and reproductive hormones, influencing poultry growth and production performance. Notably, dietary betaine has been shown to enhance egg production and quality parameters in layer birds by improving laying performance, egg mass, and oviduct index, while mitigating heat stress [3]. The improvement in laying performance can be attributed to increased estrogen and progesterone levels in the blood [3], which regulate ovulation rate and oviduct development [4]. Furthermore, betaine has been found to promote follicle-stimulating hormone and luteinizing hormone production in the anterior pituitary gland, leading to increased egg production in layer chickens [5]. In light of these findings, this study aimed to investigate the effects of different dietary betaine doses on nutrient metabolizability and egg production parameters in laying hens, providing valuable insights into the optimization of betaine supplementation in poultry nutrition.

Materials and Methods
Ethical approval
The animal experiment was conducted in accordance with guidelines approved by the Institutional Animal Ethics Committee, 12/CPCSEA Dated 12.04.2022 in the Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar.

Experimental design

This study was conducted for 16 weeks from 22 weeks old stock of laying hens up to 38 weeks which were randomly allocated into five treatment groups with 4 replicates of 8 birds in each following completely randomized design (CRD). The experimental birds in all treatment groups were fed basal diet formulated as per BIS (2007) [6] to meet energy and protein requirement. The group B0.0 was taken as control (without betaine supplementation) while in the experimental groups B0.5, B1.0, B1.5 and B2.0 betaine was supplemented @ 0.5, 1.0, 1.5 and 2.0 g/kg feed, respectively. The ingredients used in formulating basal diet and their chemical composition analyzed as per AOAC [7] are presented in Table 1. Laying hens were housed in battery cages with regular cleaning, proper ventilation, and a 16-hour photoperiod. They received feed and water ad libitum through linear feeders and waterers.

To study the balance of nitrogen and energy, a metabolism trial was conducted during 16th week of trial. One bird was randomly selected from each replicate and transferred to metabolic cages. The feed residue and excreta voided were weighed and properly recorded for final calculations of the total daily feed consumption and excreta voided. Gross energy of oven dried feed and excreta were determined by standard procedure using bomb calorimeter. Calculations were made for nitrogen retention, dry matter metabolizability and gross energy metabolizability.

The production parameters were recorded during the trial. Egg production were recorded daily, separate record for individual bird were maintained for entire experimental period i.e. 22-38 weeks of age of laying hens. Per cent hen day egg production was calculated by using following formula:

$$\text{Percent hen day egg production} = \frac{\text{Total no. of eggs produced during the period}}{\text{Total no. of hen days during the period}} \times 100$$

At the end of each week the egg weights were recorded. Egg weights were measured by using electronic weighing balance. Average egg weight of each treatment was calculated as under:

$$\text{Average egg weight (g)} = \frac{\text{Weights of all eggs of the treatment}}{\text{Total no. of eggs taken for weighing}}$$

The egg mass production was calculated using following formula:

$$\text{Egg mass production (g/day)} = \frac{\text{Percent hen day egg production}}{100} \times \text{Weight of egg}$$

The data was statistically analyzed (Snedecor and Cochran, 1994) [8] and the means of different experimental groups were tested for statistical significance.

Table 1: Ingredient and chemical composition of ration for layers

Feed ingredients	Percentage
Maize	66
Groundnut cake	7
Soybean Meal	14
Fish Meal	6
Mineral Mixture	3
Salt	0.5
Shell Grit	3.5
Chemical composition	% DM basis
Moisture	11.58
Dry matter	88.42
Crude protein	17.68
Crude fibre	3.77
Ether extract	4.55
Nitrogen free extract	69.64
Ash	4.36
Metabolizable energy*(Kcal/Kg)	2653.37

* Calculated

Results

Nutrient metabolizability

The results of the present study showed that mean values of dry matter and gross energy metabolizability were significantly (p<0.05) improved by betaine supplementation and were found highest in group B2.0 supplemented with 2g betaine per kg feed in comparison to the control (Table 2). Similarly, nitrogen retention was also seen highest (p<0.05) in group B2.0 followed by B1.5, B1.0 and B0.5 when compared to B0.0.

Table 2: Mean values of nutrients metabolizability in laying hens under different dietary treatments

Treatment	DM Metabolizability (%)	Nitrogen retention (%)	GE Metabolizability (%)
B0.0	65.85^{a}±0.21	63.87^{a}±0.18	63.46^{a}±0.19
B0.5	67.64^{b}±0.25	65.04^{b}±0.13	64.56^{b}±0.13
B1.0	67.88^{b}±0.27	65.56^{b}±0.19	64.78^{b}±0.21
B1.5	68.50^{c}±0.28	66.75^{c}±0.19	65.83^{c}±0.29
B2.0	68.59^{c}±0.24	67.74^{d}±0.32	66.84^{d}±0.26

Means bearing different superscripts in a column differ significantly (p<0.05)

Hen day egg production

The results of the present study unveiled that the percent hen day egg production mean values of betaine supplemented groups were significantly improved in comparison to the control group (p<0.05). The maximum hen day egg production percentage was seen during 30-32 weeks and the minimum was seen during 22-24 weeks of age. It was found that percent hen day egg production of groups B1.5 and B2.0 had the statistically similar but higher mean values than the other groups, with the highest overall in B2.0 group (Table 3).

Table 3: Mean values of per cent hen day egg production during progressive age (weeks) under different dietary treatments

Weeks/Treatment	B0.0	B0.5	B1.0	B1.5	B2.0
22-24	71.84ᵃ±1.64	72.11ᵃ±1.01	73.17ᵇ±1.87	73.76ᵇ±2.34	73.56ᵇ±1.32
24-26	72.11ᵃ±0.91	73.12ᵇ±0.91	73.78ᵇᶜ±0.72	74.42ᶜ±1.43	74.79ᶜ±1.23
26-28	73.22ᵃ±3.64	74.46ᵇ±0.91	74.56ᵇ±1.01	75.84ᶜ±1.01	75.51ᶜ±1.23
28-30	74.61ᵃ±0.95	75.72ᵇ±0.95	75.67ᵇ±1.20	76.23ᶜ±0.70	76.94ᶜ±1.01
30-32	75.33ᵃ±1.01	76.56ᵇ±0.45	76.94ᵇᶜ±0.95	77.56ᶜ±1.01	77.72ᶜ±0.95
32-34	74.44ᵃ±1.01	75.67ᵇ±1.01	75.89ᵇᶜ±1.64	76.78ᶜ±1.01	76.67ᶜ±0.45
34-36	73.73ᵃ±1.01	74.56ᵇ±1.01	75.94ᶜ±0.95	76.83ᶜ±0.95	76.74ᶜ±1.01
36-38	73.31ᵃ±0.70	74.40ᵇ±0.72	75.24ᵇ±0.95	76.72ᶜ±0.95	76.33ᶜ±1.01
Mean	73.57ᵃ±0.59	74.57ᵇ±0.42	75.14ᵇᶜ±0.57	76.01ᶜ±0.55	76.03ᶜ±0.46

Means bearing different superscripts in a row differ significantly (p<0.05)

Egg weight

The cumulative mean weight of eggs (gm) during progressive age of layers under different dietary treatments ranged from 51.26 (B0.0) to 53.78 (B2.0). The maximum egg weight was noticed in 2000mg/kg betaine supplemented group (B2.0) which differed significantly (p<0.05) from control group (B0.0). The highest increment in egg weight was observed during 30-32 weeks duration. The groups B1.5 and B2.0 had statistically (p>0.05) similar egg weights during all 8 fortnightly recordings.

Table 4: Mean values of egg weight (g) during progressive age (weeks) under different dietary treatments

Weeks/Treatment	B0.0	B0.5	B1.0	B1.5	B2.0
22-24	46.98ᵃ±0.28	46.92ᵃ±0.40	47.71ᵇ±0.36	48.06ᵇᶜ±0.33	48.65ᶜ±0.35
24-26	47.89ᵃ±0.58	48.58ᵃ±0.24	49.49ᵇ±0.38	51.23ᶜ±0.31	52.50ᶜ±0.30
26-28	50.98ᵃ±0.31	51.52ᵃ±0.22	52.87ᵇ±0.62	52.70ᵇ±0.75	53.82ᶜ±0.44
28-30	52.81ᵃ±0.42	53.43ᵃ±0.29	54.71ᵇ±0.45	54.65ᵇ±0.54	54.76ᵇ±0.42
30-32	53.18ᵃ±0.24	54.81ᵇ±0.31	55.60ᵇᶜ±0.52	55.58ᵇᶜ±0.32	56.18ᶜ±0.55
32-34	53.04ᵃ±0.26	54.50ᵇ±0.43	54.44ᵇ±0.31	55.66ᶜ±0.35	55.94ᶜ±0.31
34-36	52.82ᵃ±0.30	53.72ᵇ±0.44	54.14ᵇ±0.39	54.46ᵇ±0.75	54.59ᵇ±0.36
36-38	52.44ᵃ±0.39	53.49ᵇ±0.38	53.41ᵇ±0.29	54.34ᶜ±0.44	53.84ᶜ±0.37
Mean	51.26ᵃ±0.25	52.12ᵇ±0.32	52.79ᵇᶜ±0.34	53.33ᶜ±0.34	53.78ᶜ±0.37

Means bearing different superscripts in a row differ significantly (p<0.05)

Egg mass production

Our results showed that egg mass production was significantly (p<0.05) increased in laying hens groups fed diets supplemented with different doses of betaine in comparison to the control group. The maximum egg mass production was noticed during 30-32 weeks and the minimum during 22-24 weeks age (Table 5).

Table 5: Mean values of egg mass production (g/day/hen) during progressive age (weeks) under different dietary treatments

Weeks/Treatment	B0.0	B0.5	B1.0	B1.5	B2.0
22-24	33.75ᵃ±0.85	33.83ᵃ±0.28	34.90ᵇ±1.17	35.45ᵇ±1.20	35.78ᵇ±0.47
24-26	34.53ᵃ±0.45	35.52ᵃ±0.35	36.51ᵇ±0.45	38.12ᶜ±0.58	39.26ᶜ±0.42
26-28	37.32ᵃ±2.03	38.36ᵇ±0.57	39.41ᵇᶜ±1.00	39.96ᶜ±0.12	40.63ᶜ±0.37
28-30	39.40ᵃ±0.70	40.45ᵇ±0.34	41.39ᵇ±0.42	41.65ᵇᶜ±0.73	42.13ᶜ±0.27
30-32	40.06ᵃ±0.42	41.96ᵇ±0.46	42.77ᶜ±0.44	43.10ᶜ±0.79	43.66ᶜ±0.79
32-34	39.48ᵃ±0.36	41.24ᵇ±0.85	41.31ᵇ±0.67	42.73ᶜ±0.82	42.88ᶜ±0.38
34-36	38.94ᵃ±0.33	40.05ᵇ±0.86	41.11ᵇ±0.67	41.84ᶜ±0.89	41.89ᶜ±0.36
36-38	38.44ᵃ±0.30	39.79ᵇ±0.49	40.18ᵇ±0.58	41.68ᶜ±0.82	41.09ᶜ±0.32
Mean	37.74ᵃ±0.41	38.90ᵇ±0.41	39.69ᵇ±0.50	40.56ᶜ±0.47	40.91ᶜ±0.43

Means bearing different superscripts in a row differ significantly (p<0.05)

Discussion

This study revealed that the highest nutrient metabolizability was achieved in the group supplemented with betaine at a dose rate of 2000 mg/kg feed, followed closely by the lower dose groups. These findings align with previous research demonstrating enhanced nutrient digestibility with betaine supplementation. Previous studies have reported significant improvements in crude protein digestion coefficients with betaine supplementation. For instance, Ezzet *et al.* [9] observed a notable increase in crude protein digestibility when layer diets were supplemented with 1g/kg betaine. Similarly, Attia *et al.* [3] found that adding 1000 mg/kg betaine to laying hen diets resulted in significant improvements in crude protein digestibility, with no notable effects on crude fiber, dry matter, or ether extract digestibility. Betaine's osmolytic properties are thought to contribute to its beneficial effects on nutrient digestibility. By supporting intestinal cell growth and enhancing cell activity, betaine may increase nutrient absorption [10]. Furthermore, betaine's stress-reducing properties, mediated by its osmoprotective function in the intestine, may lead to improved gut morphology and function, ultimately enhancing nutrient absorption [11].

This study demonstrated that 2g/kg betaine supplementation had the most profound impact on egg production parameters, surpassing all other graded levels of betaine and the control group. Notably, the peak improvements in egg weight, hen-day egg production, and egg mass production occurred between 30-32 weeks of age. Our findings are supported by previous research, including Zou *et al.* [12] who found that betaine, in combination with thyroprotein, enhanced laying performance in hens. Ryu *et al.* [13] also found that egg production rates in hens fed 500 ppm, 1000 ppm, or 2000 ppm betaine were statistically higher than in the control group. Similarly, Zou and Feng [5] reported that 0.1% betaine supplementation increased egg production by 10%. In another study, Gudev *et al.* [14] reported that 0.7g/kg and 1.5g/kg levels of betaine increased daily egg production and egg mass in laying hens. The underlying mechanism of betaine's positive effect on egg production may be attributed to its stimulatory effect on the anterior pituitary gland, leading to increased secretion and release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These hormones promote follicle growth and ovulation, inhibit follicular atresia, and subsequently increase egg cell and egg production. Additionally, betaine's role in protein and energy metabolism may contribute to improved synthesis of egg yolk and albumen precursors, leading to enhanced egg weight and egg mass production [15]. This is consistent with findings by Xing and Jiang [16] that layers fed a 0.08% betaine diet showed significantly higher egg production than those fed a control diet. Similarly, Lu and Zou [17] found that 600 mg/kg betaine supplementation improved egg production by 8.67% compared to the control group. However, Park and Kim [18] reported no significant improvement in egg production (p>0.05) with betaine supplementation up to 1.2 g/kg, which contradicts the findings of the present study.

Conclusion

This study conclusively demonstrates the positive impact of betaine supplementation on nutrient metabolizability and egg production parameters in laying hens. The results show that 2g/kg betaine supplementation achieved the highest nutrient metabolizability and had the most profound impact on egg production parameters, including egg weight, hen-day egg production and egg mass production. These findings are supported by previous research and suggest that betaine's osmolytic and stress-reducing properties contribute to its beneficial effects on nutrient digestibility and egg production. The study's results have important implications for the poultry industry, highlighting the potential of betaine supplementation to improve the efficiency and productivity of laying hen operations. Furthermore, the findings suggest that betaine may be a valuable feed additive for enhancing the nutritional value and productivity of laying hens.

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