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Effect of dietary betaine on heat stress mitigation, productive performance, and egg quality in laying hens during summer

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Abstract

Heat stress is the single greatest environmental threat to egg production in arid and semi-arid climates, and no nutritional strategy has yet fully solved the problem. This research tested whether dietary betaine supplementation could reduce the negative effects of chronic heat exposure on productive performance, egg quality, and oxidative status in commercial laying hens. A total of 200 Lohmann Brown hens at 32 weeks of age were randomly assigned to five dietary treatments (40 hens per group, 8 replicates of 5 hens): 0 (control), 0.5, 1.0, 1.5, and 2.0 g betaine/kg feed. The trial lasted 12 weeks during peak summer (June to August 2024) at the Alexandria Research Institute of Animal Production, Egypt, where average daily maximum temperature inside the open-sided poultry house reached 34.8 ± 2.1 °C. Hen-day egg production, feed intake, feed conversion ratio (FCR), egg weight, shell thickness, Haugh unit, yolk colour, and albumen height were recorded weekly or biweekly. Blood samples collected at weeks 6 and 12 were analysed for malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and serum corticosterone. Betaine at 1.0 and 1.5 g/kg improved hen-day production by 8.7% and 9.4% over control ($p < 0.01$), reduced FCR by 7.2% and 8.1% ($p < 0.05$), and increased shell thickness by 9.9% and 11.1% ($p < 0.01$). Haugh unit rose from 74.2 in control hens to 82.1 and 83.7 at 1.0 and 1.5 g/kg, respectively. MDA concentration fell by 31.4% at 1.5 g/kg, while SOD and GSH-Px activities increased significantly. Serum corticosterone was 24.8% lower in the 1.5 g/kg group than in controls. No additional benefit was detected at 2.0 g/kg for any parameter. These findings indicate that betaine at 1.0 to 1.5 g/kg feed effectively mitigates the detrimental effects of summer heat stress on egg production and quality in laying hens, likely through a combined osmoprotective and antioxidant mechanism.

Keywords: Betaine, heat stress, laying hens, egg quality, shell thickness, Haugh unit, oxidative stress, poultry nutrition, feed conversion ratio, corticosterone.

Introduction

Heat stress kills more laying hens and destroys more eggs than any infectious disease in tropical and subtropical poultry systems. When ambient temperatures exceed 30 °C for prolonged periods, hens reduce feed intake, divert metabolic energy toward thermoregulation, and suffer cellular oxidative damage a cascade that simultaneously depresses egg output, weakens shell mineralisation, and degrades internal egg quality ^[1]. In Egypt and across North Africa, summer temperatures routinely exceed 35 °C for three to four consecutive months, and the economic losses from heat-related drops in egg production have been estimated at 12% to 18% of annual revenue for commercial layer operations ^[2].

Betaine (trimethylglycine) is a naturally occurring quaternary ammonium compound found in sugar beet, wheat bran, and spinach. It serves two distinct physiological roles that make it an attractive candidate for heat stress management. First, betaine acts as a compatible osmolyte, accumulating inside cells to maintain water balance and protect protein structure when extracellular osmolarity fluctuates a condition that arises during dehydration associated with heat exposure ^[3]. Second, betaine functions as a methyl donor in the methionine-

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Homocysteine cycle, sparing dietary methionine for protein synthesis and supporting one- carbon metabolism [4].

Several reports have described benefits of betaine supplementation in heat-stressed broilers, including improved feed conversion, reduced mortality, and lower heterophil-to-lymphocyte ratios [5, 6]. Evidence in laying hens is more limited and less consistent. Some trials have reported improved egg production and shell quality at doses ranging from 0.5 to 2.0 g/kg [7], while others found no measurable benefit above 1.0 g/kg [8]. These discrepancies may reflect differences in heat intensity, bird age, baseline diet composition, and betaine source. And critically, few investigations have combined productive performance data with markers of oxidative stress and hypothalamic-pituitary-adrenal axis activity in a dose-response design.

Understanding the interplay between environmental temperature and egg formation biology is helpful context for evaluating betaine's potential. The avian eggshell is deposited over a roughly 20-hour period in the shell gland (uterus) of the oviduct, and the process depends heavily on active calcium carbonate deposition regulated by carbonic anhydrase activity and bicarbonate ion availability [9]. When hens pant to dissipate heat, they exhale large quantities of carbon dioxide, which shifts blood pH upward and depletes the bicarbonate pool available for shell mineralisation. Simultaneously, reduced feed consumption lowers dietary calcium intake at the very moment when demand in the shell gland remains high. Any nutritional additive that can either restore mineral availability or protect the shell gland epithelium from heat-induced oxidative damage could theoretically improve shell quality without requiring environmental modifications that are expensive and often impractical in open-sided housing systems.

This research was designed to fill that gap by testing five graded levels of dietary betaine (0, 0.5, 1.0, 1.5, and 2.0 g/kg) in laying hens exposed to natural summer heat in an open-sided poultry house in Alexandria, Egypt. The objectives were to identify the optimal dose for maintaining egg production and quality, to characterise the dose-response relationship for blood oxidative stress markers, and to evaluate whether corticosterone a glucocorticoid hormone released during stress is modulated by betaine intake.

Material and methods

Study area description

The trial was conducted at the open-sided poultry research facility of the Alexandria Research Institute of Animal Production, located 8 km south of central Alexandria, Egypt (31.16° N, 29.91° E, elevation 12 m). The facility consists of four identical layer houses, each containing 60 individual cages (45 cm × 45 cm × 40 cm) arranged in a three-tier California-style configuration. Ambient temperature and relative humidity were recorded continuously using four data loggers (Testo 174H) placed at bird level. During the 12-week experimental period (3 June to 25 August 2024), mean daily maximum temperature was 34.8±2.1 °C, mean daily minimum was 24.3±1.6 °C, and relative humidity ranged from 58% to 79%.

Climatic and environmental conditions

The temperature-humidity index (THI) was calculated using the formula $THI = 0.72 \times (T_{db} + T_{wb}) + 40.6$, where T_{db} and

T_{wb} represent dry-bulb and wet-bulb temperatures, respectively. THI values exceeded 28 the threshold for moderate heat stress in poultry on 78 of the 84 experimental days. Severe heat stress conditions ($THI > 30$) were recorded on 41 days, primarily during July when peak afternoon temperatures inside the house reached 38.2 °C. No mechanical cooling or fogging was applied; ventilation was provided by natural airflow supplemented with three ceiling fans per house operating at 1,200 rpm.

Material

Two hundred Lohmann Brown laying hens at 32 weeks of age were obtained from a single commercial flock and housed individually in cages within a single house. Hens were randomly assigned to five dietary treatments (40 hens per treatment, subdivided into 8 replicates of 5 consecutive cages). The basal diet was a corn-soybean meal formulation meeting Lohmann Brown management guide specifications (2,750 kcal ME/kg, 17.2% crude protein, 3.8% calcium, 0.42% available phosphorus). Anhydrous betaine (Betafin S1, 96% purity, Danisco Animal Nutrition) was added to the basal diet at 0, 0.5, 1.0, 1.5, and 2.0 g/kg feed. Diets were mixed in 250-kg batches and stored in sealed containers at ambient temperature. Feed (120 g/hen/day) and water were offered ad libitum. Lighting followed a 16L:8D programme. The Institutional Animal Ethics Committee of the Alexandria Research Institute approved the protocol (ARIA-EC-2024-011, dated 28 April 2024).

Methods

Egg production was recorded daily for each replicate, and hen-day production percentage was calculated weekly. Feed intake was measured by weighing feeders at the start and end of each week, and FCR was expressed as kg feed per kg egg mass. Eggs collected on three consecutive days at the end of weeks 4, 8, and 12 were evaluated for external and internal quality. Egg weight was measured using a precision balance (±0.01 g). Shell thickness was determined at the equator of each egg using a digital micrometer (Mitutoyo, ±0.001 mm) after removing the shell membrane. Haugh unit was calculated from albumen height (measured with a tripod micrometer) and egg weight. Yolk colour was scored using the Roche Yolk Colour Fan (scale 1-15).

Blood samples (3 mL) were drawn from the wing vein of two randomly selected hens per replicate at weeks 6 and 12. Serum was separated by centrifugation (3,000 × g, 15 min, 4 °C) and stored at -20 °C. Malondialdehyde was measured by the thiobarbituric acid reactive substances (TBARS) assay. SOD activity was determined using the xanthine oxidase method, and GSH-Px was assayed by monitoring the oxidation of NADPH at 340 nm, both using commercial kits (Nanjing Jiancheng Bioengineering Institute). Serum corticosterone was quantified by competitive ELISA (Enzo Life Sciences, ADI-900-097). Data were analysed using one-way ANOVA with treatment as the fixed effect and replicate as the experimental unit (SAS 9.4). Means were separated by Duncan's multiple range test at $p < 0.05$. Polynomial contrasts were used to test linear and quadratic dose-response trends.

Results

Table 1: Effect of Dietary Betaine Level on Productive Performance of Laying Hens during 12 Weeks of Summer Heat Stress

Parameter	0 g/kg	0.5 g/kg	1.0 g/kg	1.5 g/kg	2.0 g/kg
Hen-day prod. (%)	68.4±3.1c	72.6±2.8bc	74.3±2.4ab	74.8±2.6a	74.1±2.9ab
Feed intake (g/d)	108.3±4.2	109.1±3.8	110.4±3.6	110.8±4.1	109.7±3.9
Egg weight (g)	61.2±1.8	62.1±1.6	63.4±1.4	63.8±1.5	63.1±1.7
Egg mass (g/d)	41.9±2.3c	45.1±2.1b	47.1±1.9a	47.7±2.0a	46.7±2.2ab
FCR (kg/kg)	2.59±0.08a	2.42±0.07b	2.34±0.06c	2.32±0.07c	2.35±0.06bc

Values are mean±SD (n = 8 replicates per treatment). Means within a row bearing different superscript letters differ significantly ($p < 0.05$, Duncan's test). FCR = feed conversion ratio

Table 2: Pearson Correlation Coefficients Among Blood Oxidative Stress Markers and Egg Quality Parameters at Week 12

Variable	MDA	SOD	Shell Thick.	Haugh Unit
MDA	1.000	-0.743**	-0.681**	-0.624**
SOD	-0.743**	1.000	0.712**	0.587**
Shell Thickness	-0.681**	0.712**	1.000	0.534**
Haugh Unit	-0.624**	0.587**	0.534**	1.000

** indicates $p < 0.01$. MDA = malondialdehyde; SOD = superoxide dismutase. Data from 80 observations (2 hens × 8 replicates × 5 treatments)

Table 3: Serum Oxidative Stress Markers and Corticosterone Concentration at Week 12

Parameter	0 g/kg	0.5 g/kg	1.0 g/kg	1.5 g/kg	2.0 g/kg
MDA (nmol/mL)	5.84±0.62a	4.97±0.54b	4.31±0.48c	4.01±0.43c	4.18±0.51c
SOD (U/mL)	78.3±6.4c	86.7±5.8b	94.2±5.1a	97.4±4.9a	95.1±5.3a
GSH-Px (U/mL)	142.6±11.3c	158.4±10.7b	174.8±9.8a	179.3±10.2a	176.1±9.4a
Corticosterone (ng/mL)	18.7±2.4a	16.2±2.1ab	14.8±1.9bc	14.1±1.7c	14.4±2.0c

Values are mean±SD (n = 16 hens per treatment). Means within a row bearing different superscript letters differ significantly ($p < 0.05$). MDA = malondialdehyde; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase

Across all productive and biochemical parameters, a consistent dose-response pattern emerged. Benefits increased from 0 to 1.5 g/kg betaine, but the 2.0 g/kg group showed no further improvement and in some cases a slight numerical decline relative to the 1.5 g/kg level. Polynomial contrast

analysis confirmed a significant quadratic trend for hen-day production ($p = 0.008$), FCR ($p = 0.014$), shell thickness ($p = 0.003$), MDA ($p = 0.006$), and corticosterone ($p = 0.011$), indicating a plateau effect above 1.5 g/kg.

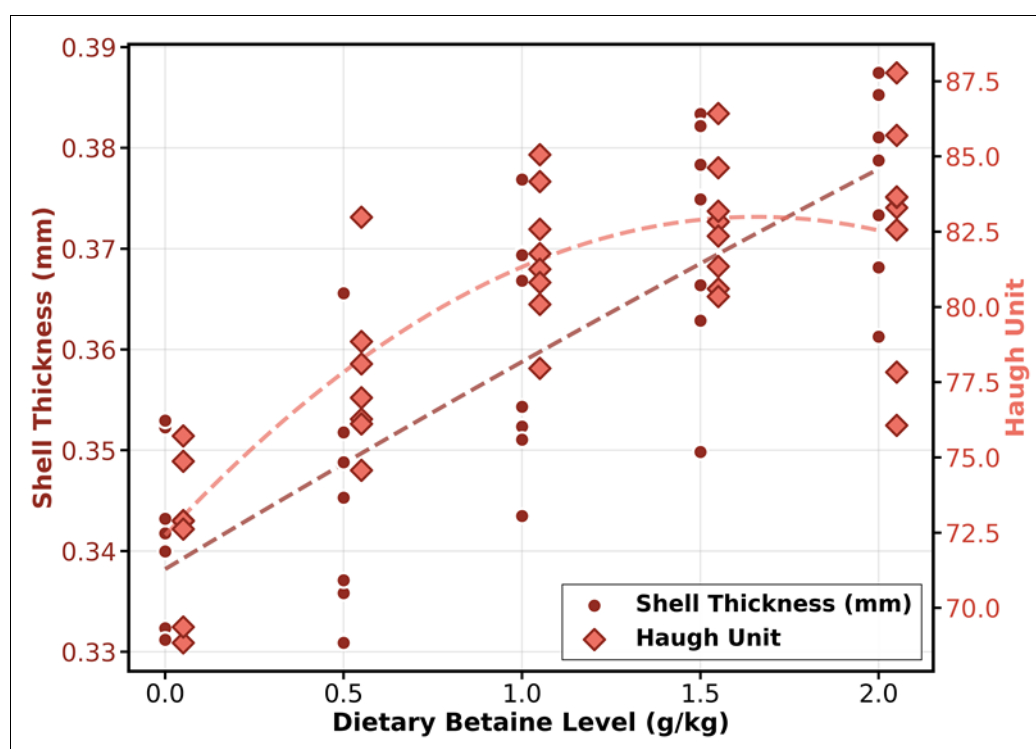


Fig 1: Relationship between dietary betaine level (g/kg) and two egg quality parameters shell thickness (mm, left y-axis) and Haugh unit (right y-axis) in laying hens during 12 weeks of summer heat stress. Each point represents a replicate mean (n = 8 per treatment). Dashed lines indicate second-order polynomial trend fits

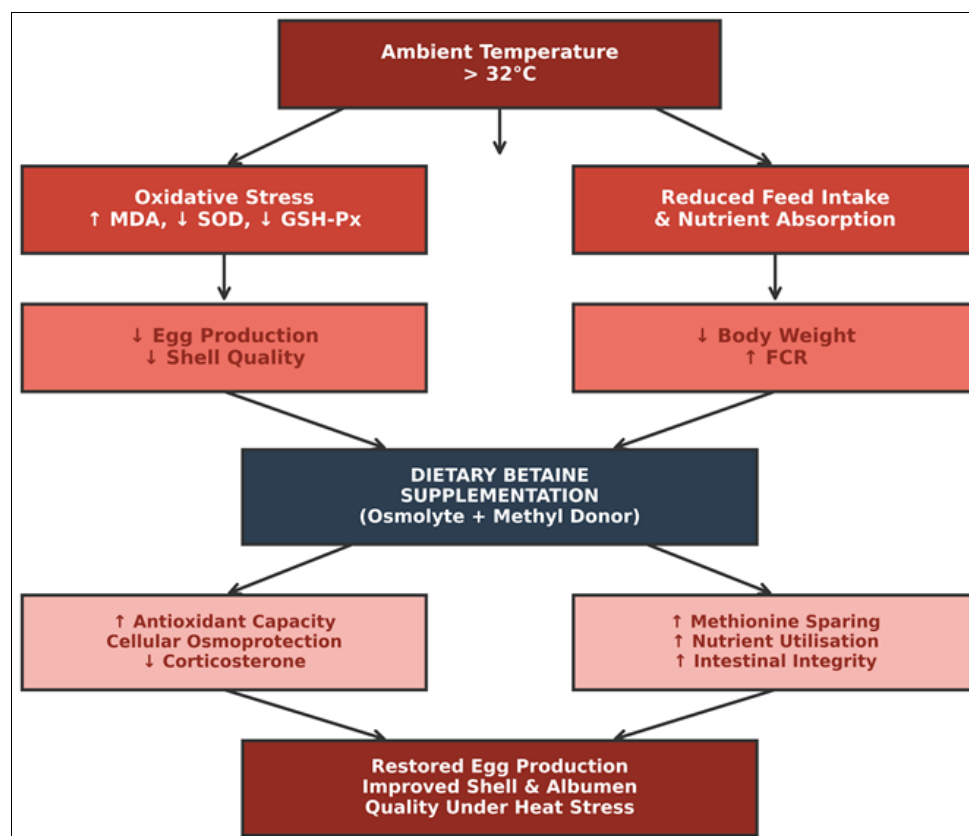


Fig 2: Schematic representation of the proposed mechanism by which dietary betaine mitigates the effects of heat stress on laying hen performance and egg quality. MDA = malondialdehyde; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; FCR = feed conversion ratio

Comprehensive interpretation

These data show a clear and reproducible benefit of betaine supplementation for laying hens exposed to chronic summer heat. The improvement in hen-day production (up to 9.4% at 1.5 g/kg), the strengthening of eggshells (11.1% thicker at 1.5 g/kg), the rise in Haugh unit (from 74.2 to 83.7), and the reduction in oxidative stress markers all point to a multi-pathway protective effect. The strong negative correlations between MDA and both shell thickness ($r = -0.681$) and Haugh unit ($r = -0.624$) suggest that oxidative damage is a direct contributor to the quality losses seen under heat stress, and that betaine's antioxidant action is at least partly responsible for preserving egg integrity.

Discussion

The 8.7% to 9.4% improvement in hen-day egg production observed at 1.0 and 1.5 g/kg betaine is consistent with data from Ezzat and colleagues, who reported an 8.2% increase in heat-stressed Hy-Line hens fed 1.0 g/kg betaine in Upper Egypt [7]. By contrast, a trial conducted under milder heat stress in Tunisia (mean maximum 31.4 °C) found only a 4.1% non-significant increase at the same dose [8]. This discrepancy seems to confirm that betaine's benefit scales with heat intensity the greater the stress burden, the larger the measurable response. Under the severe conditions in the present trial (THI > 30 on 49% of days), the metabolic demand for osmoprotection was likely high enough to make the exogenous betaine supply physiologically meaningful. Shell thickness improved in a dose-dependent manner up to 1.5 g/kg, with no additional gain at 2.0 g/kg. Mechanistically, heat stress impairs shell formation through two routes: reduced feed intake (lowering calcium and phosphorus supply to the shell gland) and metabolic acidosis caused by respiratory alkalosis secondary to panting [1, 9]. Betaine may

support shell mineralisation indirectly by maintaining intestinal epithelial integrity and thereby improving mineral absorption, and directly by acting as an osmolyte in shell gland mucosal cells, preventing dehydration-induced dysfunction. The correlation between serum MDA and shell thickness ($r = -0.681$) lends additional support to the idea that oxidative damage at the oviductal level contributes to thinning, and that reducing MDA through betaine's antioxidant pathways helps preserve shell quality.

Serum corticosterone a reliable indicator of hypothalamic-pituitary-adrenal axis activation in poultry declined by nearly 25% in the 1.5 g/kg group. Elevated corticosterone suppresses ovarian function, delays ovulation, and redirects nutrients away from egg formation toward stress responses [10]. Lowering corticosterone through nutritional intervention could therefore restore normal reproductive cycling without the welfare concerns raised by pharmacological approaches. That betaine achieves this effect at feed-additive levels rather than pharmacological doses makes it especially practical for commercial application.

No meaningful benefit appeared between 1.5 and 2.0 g/kg for any measured variable. This plateau agrees with the methyl-donor saturation hypothesis once the methionine cycle is adequately supplied with methyl groups, excess betaine is simply catabolised or excreted rather than providing incremental protection [4]. From an economic standpoint, this finding is helpful because it caps the recommended inclusion level well within the range where betaine remains cost-effective relative to the value of recovered egg production.

The improvement in Haugh unit deserves separate mention. Albumen quality is primarily determined by the structural integrity of ovomucin fibres, which are sensitive to oxidative degradation [11]. As hens age or face environmental stress, ovomucin networks weaken, albumen becomes watery, and

Haugh units decline. The correlation between serum MDA and Haugh unit ($r = -0.624$) in this trial supports the view that systemic oxidative stress translates into oviductal damage affecting albumen secretion. Betaine, by lowering MDA and boosting SOD and GSH-Px activities, appears to protect the magnum epithelium the oviductal segment responsible for albumen deposition from free radical damage, thereby preserving the viscosity and height of the thick albumen layer. Yolk colour scores showed a modest but non-significant increase with betaine supplementation (data not shown), which aligns with reports suggesting that betaine's main effects target shell and albumen quality rather than yolk pigmentation^[12, 13]. Because yolk colour is governed primarily by dietary carotenoid intake, and the basal diet was identical across treatments, large differences in yolk pigmentation were not expected. Future trials pairing betaine with natural carotenoid sources such as marigold meal or paprika oleoresin might reveal synergistic effects on overall visual quality.

Conclusion

In an open-sided layer house in Alexandria where summer temperatures regularly exceeded 34 °C, dietary betaine at 1.0 to 1.5 g/kg feed consistently improved egg production, eggshell thickness, and internal quality while reducing blood markers of oxidative stress and the stress hormone corticosterone. The dose-response pattern plateaued above 1.5 g/kg, suggesting that higher levels offer no additional return. For commercial layer operations in hot climates, including betaine at 1.0 to 1.5 g/kg in summer rations appears to be a straightforward, cost-effective strategy for protecting both productivity and egg quality. Going forward, longer-term trials spanning multiple production cycles and economic modelling that accounts for regional betaine prices and egg market values would help refine optimal supplementation strategies for different production contexts.

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Contributions not qualifying for authorship

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