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Effects of dietary vitamin C, betaine, organic selenium and their combination on physiological responses, growth performance, carcass traits and intestinal morphology in arbor acres broiler chickens

Abstract

Heat stress poses a major challenge to broiler production in semi-arid regions, impairing growth performance, carcass quality and physiological homeostasis. This study evaluated the effects of dietary supplementation with vitamin C (250 mg/kg), betaine (2 g/kg), organic selenium (0.3 mg/kg as selenomethionine) and their combination, provided throughout the 42-day rearing period, on Arbor Acres broilers exposed to natural semi-arid heat stress (34–40 °C, 20–45 % relative humidity) at Ahmadu Bello University, Zaria, Nigeria. Three hundred one-day-old male chicks were randomly assigned to five treatments in a completely randomised design with four replicates each. The combination treatment significantly reduced rectal temperature (40.8–40.9 °C compared to 41.5–41.8 °C in control), heterophil to lymphocyte ratio (0.28 compared to 0.52), serum corticosterone (11.4 ng/ml compared to 18.6 ng/ml) and malondialdehyde (2.9 nmol/ml compared to 5.2 nmol/ml), while increasing superoxide dismutase (17.3 U/ml compared to 12.4 U/ml) and glutathione peroxidase (38.7 U/ml compared to 28.1 U/ml) activities ($P < 0.05$). Final body weight (2410 g compared to 2105 g) and feed conversion ratio (1.65 compared to 1.83) improved markedly, alongside higher dressing percentage (76.2 % compared to 72.4 %), breast yield (28.9 % compared to 25.8 %) and jejunal villus height (1165 µm compared to 895 µm) and villus: crypt ratio (7.37 compared to 4.84). Individual supplements provided intermediate benefits, with the combination demonstrating clear synergistic effects on antioxidant defence, stress attenuation, nutrient utilisation and intestinal integrity. These findings indicate that combined nutritional modulation effectively alleviates heat stress physiology and sustains productivity in semi-arid broiler production systems.

Keywords: heat stress, broiler chickens, vitamin C, betaine, organic selenium, semi-arid conditions, antioxidant defence, intestinal morphology, growth performance

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Introduction

Broiler chickens produce substantial metabolic heat owing to their accelerated growth rates, rendering them particularly vulnerable to ambient temperatures exceeding the thermo neutral zone in semi-arid environments.[1] Heat stress triggers panting as the predominant evaporative cooling mechanism, which frequently results in respiratory alkalosis and consequent disruptions in blood pH and electrolyte equilibrium. Voluntary feed intake decreases significantly as birds divert resources toward thermoregulation rather than nutrient acquisition, thereby limiting energy for growth and physiological maintenance.[2] Oxidative stress escalates through heightened generation of reactive oxygen species that surpass antioxidant defences and inflict damage on lipids, proteins and DNA.[3] Intestinal barrier function deteriorates with reduced villus height and elevated permeability, promoting bacterial translocation and systemic inflammatory responses.[4] Immune competence declines, as indicated by increased heterophil to lymphocyte ratios and diminished antibody titres.[5] Carcass characteristics worsen, including greater prevalence of pale, soft, exudative meat and lower breast meat proportions.[6] Mortality intensifies during acute thermal episodes when daytime temperatures surpass 35 °C alongside low relative humidity.[7] Substantial economic repercussions emerge from impaired body weight gain, elevated feed conversion ratios and augmented management expenditures in impacted flocks.[8]

Semi-arid climates in northern Nigeria, including the Zaria region, subject poultry to extended periods of elevated temperatures, with maxima often exceeding 38 °C during March to May, thereby aggravating physiological strain in commercial broiler operations.[9] Nigeria has an estimated standing poultry population of approximately 180 million birds, contributing significantly to the agricultural sector (accounting for about 25% of agricultural GDP in some estimates) and providing an important source of animal protein for its growing human population (Poultry Association of Nigeria, 2025). Arbor Acres broilers, a widely utilised commercial strain, display notable sensitivity to such thermal loads, culminating in compromised performance unless nutritional countermeasures are applied.[10] Nutritional regulation constitutes a practical, economically viable strategy to ameliorate stress physiology by bolstering antioxidant capacity, preserving osmotic homeostasis and reinforcing intestinal integrity.[11] Vitamin C supplementation augments cellular protection against oxidative insult and moderates hormonal perturbations during thermal exposure.[12] Betaine functions as a potent osmoprotectant that sustains cellular hydration and enhances intestinal villus architecture under heat load.[13] Organic selenium elevates glutathione peroxidase activity and attenuates lipid peroxidation in vital tissues.[3] Combined

provision of these nutrients elicits synergistic effects that concurrently target multiple heat stress pathways.[4]

The core issue resides in recurrent physiological impairments induced by heat stress that undermine broiler productivity, welfare and meat quality in semi-arid settings devoid of sufficient nutritional fortification. Scant experimental evidence exists specifically for combined dietary interventions employing vitamin C, betaine and organic selenium in Arbor Acres broilers within Nigerian semi-arid contexts, impeding refined feed formulation approaches.[4] The rationale for this investigation arises from the pressing requirement to delineate efficacious nutritional interventions that uphold performance amid escalating climatic variability whilst diminishing dependence on costly environmental controls.[13] The objective of the present study was to assess the impacts of dietary vitamin C, betaine, organic selenium and their combination on physiological indices, growth performance, carcass attributes and intestinal morphology in Arbor Acres broiler chickens subjected to natural semi-arid heat stress at Ahmadu Bello University, Zaria.

Materials and methods

The experiment was conducted at the Poultry Research Unit, Ahmadu Bello University, Zaria, Kaduna State, Nigeria (latitude 11°04′N, longitude 7°42′E), a representative semi-arid locale featuring daytime temperatures averaging 34–40 °C and relative humidity of 20–45 % throughout the March–May 2025 experimental duration (IARMS, 2025). All protocols gained approval from the Ahmadu Bello University Animal Ethics Committee (Approval number: ABUCAUC/2024/147) and conformed to institutional poultry welfare standards.

Three hundred one-day-old male Arbor Acres broiler chicks were acquired from Fidan Hatchery, Ibadan, Oyo State, Nigeria. Upon receipt, chicks underwent vaccination against Newcastle disease on day 1 and infectious bursal disease on day 14. Initial individual body weights were recorded, followed by random allocation to five dietary treatments in a completely randomised design (CRD). Each treatment comprised 60 birds divided into four replicate pens containing 15 birds apiece, yielding a stocking density of 10 birds per m². Pens dimensions were 2 m × 1.5 m, equipped with concrete flooring overlaid by 10 cm wood shavings litter. Ventilation relied on natural airflow augmented by ceiling fans to facilitate air circulation absent artificial cooling.

The basal maize-soybean meal diet satisfied or surpassed nutrient specifications for Arbor Acres broilers in three phases: starter (days 1–21: 22 % crude protein, 3000 kcal metabolisable energy/kg) and finisher (days 22–42: 18 % crude protein, 3200 kcal metabolisable energy/kg) as shown in Tables 1 and 2 respectively. Treatments consisted of: (1) basal diet alone (control); (2) basal diet plus vitamin C (L-ascorbic acid) at 250 mg/kg; (3) basal diet plus betaine hydrochloride at 2 g/kg; (4) basal diet plus organic selenium (hydroxy-selenomethionine, Selisseo®) at 0.3 mg/kg; and (5) basal diet plus the combination of vitamin C at 250 mg/kg, betaine at 2 g/kg and organic selenium at 0.3 mg/kg.[3,11,13]

Supplements were uniformly blended into the diets using a horizontal ribbon mixer. The small inclusion rate of organic selenium (0.3 mg Se/kg diet) was achieved by first preparing a concentrated premix (10 g Selisseo® per kg of carrier) that was then incorporated into the final diet to ensure homogeneous distribution and avoid any risk of localised high concentrations that could be toxic. Feed and potable water were supplied *ad libitum* across the 42-day period.

The actual concentrations of supplemental vitamin C and selenium in the finished feeds were not analysed in this study. However, supplements were added based on manufacturer specifications and standard inclusion rates reported in the literature.[3,11,13] Future studies may include feed analysis to confirm delivered doses (Table 1&2).

Table 1 Ingredient composition (g/kg, as-fed basis) and calculated nutrient composition of the experimental starter diets (days 1–21)

Ingredient (%)	Control	Vitamin C	Betaine	Organic Se	Combination
Maize	565	564.75	563	564.7	562.45
Soybean meal (48 % CP)	355	355	355	355	355
Soybean oil	40	40	40	40	40
Limestone	12	12	12	12	12
Dicalcium phosphate	15	15	15	15	15
Common salt	3.5	3.5	3.5	3.5	3.5
Vitamin premix[1]	2.5	2.5	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5	2.5	2.5
DL-Methionine	2.5	2.5	2.5	2.5	2.5
L-Lysine HCl	2	2	2	2	2
Vitamin C (L-ascorbic acid)	0	0.25	0	0	0.25
Betaine hydrochloride	0	0	2	0	2
Organic selenium[3]	0	0	0	0.3	0.3
Total	**1000**	**1000**	**1000**	**1000**	**1000**
Calculated analysis (%)					
Crude protein	22	22	22	22	22
Ether extract	6.8	6.8	6.8	6.8	6.8
Crude fibre	4.2	4.2	4.2	4.2	4.2
Calcium	0.95	0.95	0.95	0.95	0.95
Available phosphorus	0.45	0.45	0.45	0.45	0.45
Lysine	1.25	1.25	1.25	1.25	1.25
Methionine	0.55	0.55	0.55	0.55	0.55
Metabolizable energy (Kcal/kg)	3000	3000	3000	3000	3000

Vitamin premix supplied per kg diet: vitamin A 12 000 IU, vitamin D_3 3000 IU, vitamin E 30 IU, vitamin K_3 3 mg, vitamin B_1 2 mg, vitamin B_2 8 mg, vitamin B_6 4 mg, vitamin B_{12} 0.02 mg, niacin 50 mg, pantothenic acid 15 mg, folic acid 1.5 mg, biotin 0.15 mg. [2]Mineral premix supplied per kg diet: Mn 80 mg, Zn 60 mg, Fe 40 mg, Cu 8 mg, I 0.5 mg, Se 0.2 mg (basal level). [3]Organic selenium (selenomethionine) supplied 0.3 mg Se/kg diet.

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Table 2 Ingredient composition (g/kg, as-fed basis) and calculated nutrient composition of the experimental finisher diets (days 22–42)

Ingredient	Control	Vitamin C	Betaine	Organic Se	Combination
Maize	620.00	619.75	618.00	619.70	617.45
Soybean meal (48 % CP)	300.00	300.00	300.00	300.00	300.00
Soybean oil	45.00	45.00	45.00	45.00	45.00
Limestone	10.00	10.00	10.00	10.00	10.00
Dicalcium phosphate	14.00	14.00	14.00	14.00	14.00
Common salt	3.50	3.50	3.50	3.50	3.50
Vitamin premix[1]	2.50	2.50	2.50	2.50	2.50
Mineral premix[2]	2.50	2.50	2.50	2.50	2.50
DL-Methionine	1.80	1.80	1.80	1.80	1.80
L-Lysine HCl	1.50	1.50	1.50	1.50	1.50
Vitamin C (L-ascorbic acid)	0.00	0.25	0.00	0.00	0.25
Betaine hydrochloride	0.00	0.00	2.00	0.00	2.00
Organic selenium[3]	0.00	0.00	0.00	0.30	0.30
Total	**1000.00**	**1000.00**	**1000.00**	**1000.00**	**1000.00**
Calculated analysis					
Crude protein	18.50	18.50	18.50	18.50	18.50
Ether extract	7.50	7.50	7.50	7.50	7.50
Crude fibre	3.80	3.80	3.80	3.80	3.80
Calcium	0.85	0.85	0.85	0.85	0.85
Available phosphorus	0.42	0.42	0.42	0.42	0.42
Lysine	1.05	1.05	1.05	1.05	1.05
Methionine	0.48	0.48	0.48	0.48	0.48
Metabolizable energy (Kcal/kg)	3200.00	3200.00	3200.00	3200.00	3200.00

Vitamin premix supplied per kg diet: vitamin A 10 000 IU, vitamin D_3 2500 IU, vitamin E 25 IU, vitamin K_3 2.5 mg, vitamin B_1 1.5 mg, vitamin B_2 6 mg, vitamin B_6 3 mg, vitamin B_{12} 0.015 mg, niacin 40 mg, pantothenic acid 12 mg, folic acid 1 mg, biotin 0.1 mg. [2]Mineral premix supplied per kg diet: Mn 70 mg, Zn 50 mg, Fe 35 mg, Cu 7 mg, I 0.4 mg, Se 0.2 mg (basal level). [3]Organic selenium (selenomethionine) supplied 0.3 mg Se/kg diet.

Internal pen maximum and minimum temperatures together with relative humidity were logged daily at 1400 h utilising digital thermo-hygrometers. Rectal temperature was determined daily at 1400 h in five birds per pen via a digital thermometer inserted 3 cm into the cloaca.[7] Weekly pen-based recordings of body weight and feed intake enabled calculation of feed conversion ratio. Blood samples (3 ml) were drawn from the brachial vein of four birds per pen on days 21, 35 and 42. Serum corticosterone was assayed via commercial ELISA kits, malondialdehyde through thiobarbituric acid reactive substances methodology, superoxide dismutase and glutathione peroxidase activities employing enzymatic kits and heterophil to lymphocyte ratios from Giemsa-stained smears.[4] Plasma sodium, potassium and chloride were quantified by ion-selective electrode techniques.

Serum malondialdehyde (MDA) concentration was determined by the thiobarbituric acid reactive substances (TBARS) method as described by Oni *et al*.,[7] with results expressed as nmol/ml. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were measured using commercial enzymatic assay kits (Randox Laboratories, UK for SOD; Cayman Chemical, USA for GPx) following the manufacturers' instructions, with SOD activity expressed in U/ml (one unit defined as the amount of enzyme that inhibits the rate of cytochrome c reduction by 50% under specified conditions).

On day 42, individual body weights were measured prior to humane slaughter following electrical stunning. Assessed carcass traits encompassed dressing percentage, breast meat yield, abdominal fat weight and meat pH at 24 h post-mortem. Duodenal, jejunal and ileal segments were excised, fixed in 10 % neutral buffered formalin and subjected to histomorphometric evaluation of villus height, crypt depth and villus to crypt ratio using ImageJ software.[13] Data underwent one-way analysis of variance in SPSS version 27 software. Means were differentiated by Tukey's honestly significant difference test at $P < 0.05$. Normality and variance homogeneity were verified via Shapiro–Wilk and Levene's tests, respectively.

Results and discussion

Table 3 presents the effect of dietary treatments on rectal temperature and heterophil to lymphocyte ratio in Arbor Acres broilers under semi-arid heat stress. Rectal temperature showed a significant ($P < 0.05$) difference across treatments on days 21, 35 and 42. The combination treatment recorded the lowest rectal temperatures of 40.8 °C on day 21, 40.9 °C on day 35 and 40.8 °C on day 42. The vitamin C, betaine and organic selenium treatments produced statistically similar values ranging between 41.0 °C and 41.3 °C across the three days, while the control group exhibited the highest values of 41.5 °C, 41.8 °C and 41.7 °C respectively. The heterophil to lymphocyte ratio at day 42 displayed a significant ($P < 0.05$) difference, with the combination treatment yielding the lowest value of 0.28, followed by betaine at 0.32, organic selenium at 0.34 and vitamin C at 0.35, compared with the highest ratio of 0.52 in the control group. The lower rectal temperatures recorded in the supplemented groups demonstrate improved thermoregulation capacity under natural semi-arid heat stress. The combination treatment achieved the greatest reduction in core body temperature because vitamin C scavenges free radicals, betaine maintains cellular osmolarity and organic selenium supports enzymatic antioxidant systems, collectively minimising metabolic heat accumulation and panting intensity. Lower heterophil to lymphocyte ratios signify reduced physiological stress and preserved immune balance, which carry direct implications for lower mortality risk and

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better flock uniformity in commercial semi-arid production. These results agree with Oni et al.[7] who reported similar declines in rectal temperature when nutritional supplements were provided to broilers during hot-dry seasons in Nigeria. The findings also align with Al-Khalaifah et al.[12] who observed comparable temperature reductions with vitamin C under heat challenge, yet contrast with Wasti et al.[5] who noted less pronounced effects when single supplements were used instead of combinations.

Table 3 Effect of dietary treatments on rectal temperature (°C) and heterophil to lymphocyte (H/L) ratio in arbor acres broilers under semi-arid heat stress

Parameter	Day	Control	Vitamin C (250 mg/kg)	Betaine (2 g/kg)	Organic Se (0.3 mg/kg)	Combination	SEM	P-value
Rectal temperature	21	41.5^{a}	41.1^{b}	41.0^{b}	41.2^{b}	40.8^{c}	0.12	<0.001
Rectal temperature	35	41.8^{a}	41.2^{b}	41.1^{b}	41.3^{b}	40.9^{c}	0.14	<0.001
Rectal temperature	42	41.7^{a}	41.1^{b}	41.0^{b}	41.2^{b}	40.8^{c}	0.11	<0.001
H/L ratio	42	0.52^{a}	0.35^{b}	0.32^{b}	0.34^{b}	0.28^{c}	0.03	<0.01

^{abc} means on the same rows with different superscripts are significantly (P>0.05) different; SEM: Standard Error of Mean; P-value: Probability Value.

Table 4 shows the influence of dietary treatments on serum corticosterone, malondialdehyde, and superoxide dismutase and glutathione peroxidase at day 42. Serum corticosterone concentration differed significantly ($P < 0.05$) among treatments, with the combination treatment achieving the lowest value of 11.4 ng/ml, followed by organic selenium at 13.5 ng/ml, vitamin C at 13.8 ng/ml and betaine at 14.2 ng/ml, whereas the control recorded the highest concentration of 18.6 ng/ml. Malondialdehyde level followed a similar pattern with a significant ($P < 0.05$) difference; the combination treatment produced the lowest value of 2.9 nmol/ml, followed by organic selenium at 3.4 nmol/ml, vitamin C at 3.6 nmol/ml and betaine at 3.8 nmol/ml, in contrast to the highest value of 5.2 nmol/ml in the control. Superoxide dismutase and glutathione peroxidase activities increased significantly ($P < 0.05$) in all supplemented groups compared with the control, with the combination treatment exhibiting the highest activities of 17.3 U/ml for superoxide dismutase and 38.7 U/ml for glutathione peroxidase, while the control showed the lowest activities of 12.4 U/ml and 28.1 U/ml respectively. The marked decline in corticosterone levels in supplemented birds indicates effective modulation of the hypothalamic-pituitary-adrenal axis, thereby limiting catabolic effects of chronic heat stress. Reduced malondialdehyde coupled with elevated superoxide dismutase and glutathione peroxidase activities confirms robust protection against oxidative damage to cellular membranes and proteins. The superior response in the combination treatment arises from synergistic interactions that simultaneously quench radicals, stabilise membranes and enhance enzymatic defence. These biochemical improvements imply higher cellular integrity, reduced tissue injury and sustained metabolic efficiency in semi-arid environments. The data corroborate Shakeri et al.[3] who documented parallel decreases in corticosterone and malondialdehyde with combined vitamin C and selenium in heat-stressed broilers. Similar antioxidant enzyme elevations were reported by Kim et al.,[11] although the magnitude of response was slightly lower than the present combination effect, confirming the added benefit of betaine inclusion.

Table 4 Effect of dietary treatments on serum corticosterone (ng/ml), malondialdehyde (MDA, nmol/ml), superoxide dismutase (SOD, U/ml) and glutathione peroxidase (GPx, U/ml) in arbor acres broilers under semi-arid heat stress at day 42

Parameter	Control	Vitamin C (250 mg/kg)	Betaine (2 g/kg)	Organic Se (0.3 mg/kg)	Combination	SEM	P-value
Corticosterone (ng/ml)	18.6^{a}	13.8^{b}	14.2^{b}	13.5^{b}	11.4^{c}	0.85	<0.001
MDA (nmol/ml)	5.2^{a}	3.6^{b}	3.8^{b}	3.4^{b}	2.9^{c}	0.28	<0.001
SOD (U/ml)	12.4^{b}	15.8^{a}	15.2^{a}	16.1^{a}	17.3^{a}	0.92	<0.05
GPx (U/ml)	28.1^{b}	34.6^{a}	33.9^{a}	35.2^{a}	38.7^{a}	1.45	<0.01

^{abc} means on the same rows with different superscripts are significantly (P>0.05) different; SEM: Standard Error of Mean; P-value: Probability Value.

Table 5 reveals the effect of dietary treatments on growth performance parameters over the 42-day period. Final body weight displayed a significant ($P < 0.05$) difference, with the combination treatment attaining the highest value of 2410 g, followed by betaine at 2325 g, organic selenium at 2305 g and vitamin C at 2280 g, compared with the lowest value of 2105 g in the control. Feed conversion ratio also showed a significant ($P < 0.05$) difference; the combination treatment achieved the best value of 1.65, followed by betaine at 1.70, organic selenium at 1.71 and vitamin C at 1.72, while the control recorded the poorest value of 1.83. Total feed intake showed no significant ($P > 0.05$) difference among treatments. Higher final body weight and improved feed conversion ratio in the supplemented groups demonstrate enhanced nutrient partitioning toward growth rather than stress mitigation. The combination treatment delivered the greatest performance gains because the supplements collectively reduced energy expenditure on thermoregulation and oxidative repair while preserving intestinal absorptive capacity. These performance advantages translate to shorter production cycles, lower feed costs per kilogram of gain and increased profitability for farmers operating under semi-arid conditions. The outcomes match those of Saleh *et al.*[13] who recorded comparable body weight improvements with betaine and organic minerals in heat-stressed broilers. The results further support Olayiwola and Adedokun[4] who attributed better feed efficiency to multi-nutrient strategies, although their reported magnitude of improvement was marginally lower than the present synergistic combination.

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Table 5 Effect of dietary treatments on growth performance parameters in arbor acres broilers under semi-arid heat stress (days 1–42)

Parameter	Control	Vitamin C (250 mg/kg)	Betaine (2 g/kg)	Organic Se (0.3 mg/kg)	Combination	SEM	P-value
Final body weight (g)	2105^{c}	2280^{b}	2325^{b}	2305^{b}	2410^{a}	48.2	<0.001
Total feed intake (g/bird)	3850	3920	3955	3930	3980	62.1	0.082
Feed conversion ratio	1.83^{a}	1.72^{b}	1.70^{b}	1.71^{b}	1.65^{c}	0.04	<0.01

^{abc} means on the same rows with different superscripts are significantly (P>0.05) different; SEM: Standard Error of Mean; P-value: Probability Value.

Table 6 displays the effect of dietary treatments on carcass traits at day 42. Dressing percentage exhibited a significant ($P < 0.05$) difference, with the combination treatment yielding the highest value of 76.2 %, followed by betaine at 74.8 %, while the vitamin C and organic selenium treatments gave statistically similar intermediate values of 74.1 % and 74.5 % and the control recorded the lowest value of 72.4 %. Breast yield showed a significant ($P < 0.05$) difference, with the combination treatment producing the highest value of 28.9 %, followed by betaine at 27.5 %, vitamin C at 27.2 % and organic selenium at 27.0 %, in contrast to the lowest value of 25.8 % in the control. Abdominal fat percentage differed significantly ($P < 0.05$), with the combination treatment recording the lowest value of 1.5 %, followed by betaine at 1.7 %, while the vitamin C and organic selenium treatments produced statistically similar values of 1.8 % and the control exhibited the highest value of 2.1 %. Meat pH at 24 h post-mortem showed no significant ($P > 0.05$) difference among treatments. Elevated dressing percentage and breast yield in supplemented birds reflect greater muscle deposition and reduced carcass shrinkage associated with heat stress. The lowest abdominal fat in the combination and betaine groups indicates more efficient energy utilisation and less diversion of nutrients to fat storage. These carcass improvements enhance marketable yield and breast meat proportion, which are economically critical traits for commercial broiler enterprises in semi-arid zones. The present carcass responses are consistent with Abdel-Moneim *et al*.[14] who reported enhanced dressing and breast percentages following antioxidant and osmolyte supplementation. The reduction in abdominal fat aligns closely with findings of Madkour *et al*.,[6] although their single-supplement trials produced slightly smaller reductions than the current multi-nutrient approach.

Table 6 Effect of dietary treatments on carcass traits at day 42 in arbor acres broilers under semi-arid heat stress

Parameter	Control	Vitamin C (250 mg/kg)	Betaine (2 g/kg)	Organic Se (0.3 mg/kg)	Combination	SEM	P-value
Dressing percentage (%)	72.4^{b}	74.1^{ab}	74.8^{a}	74.5^{ab}	76.2^{a}	0.85	<0.05
Breast yield (%)	25.8^{c}	27.2^{b}	27.5^{b}	27.0^{b}	28.9^{a}	0.62	<0.01
Abdominal fat (%)	2.1^{a}	1.8^{ab}	1.7^{b}	1.8^{ab}	1.5^{b}	0.15	<0.05
Meat pH (24 h post-mortem)	5.92	5.88	5.85	5.87	5.84	0.04	0.214

^{abc} means on the same rows with different superscripts are significantly (P>0.05) different; SEM: Standard Error of Mean; P-value: Probability Value.

Table 7 presents the effect of dietary treatments on jejunal morphology at day 42. Villus height displayed a significant ($P < 0.05$) difference, with the combination treatment achieving the greatest value of 1165 µm, followed by betaine at 1080 µm, organic selenium at 1050 µm and vitamin C at 1025 µm, compared with the lowest value of 895 µm in the control. Villus to crypt ratio followed the same pattern with a significant ($P < 0.05$) difference; the combination treatment recorded the highest ratio of 7.37, followed by betaine at 6.43, organic selenium at 6.18 and vitamin C at 5.96, while the control showed the lowest ratio of 4.84. Crypt depth showed a significant ($P < 0.05$) difference, with the combination treatment yielding the lowest value of 158 µm, followed by betaine at 168 µm and organic selenium at 170 µm, while the vitamin C treatment gave a statistically similar value of 172 µm and the control recorded the highest value of 185 µm. Increased villus height and villus to crypt ratio together with decreased crypt depth in supplemented groups signify maintenance of intestinal epithelial integrity and absorptive surface area under thermal stress. The superior morphology in the combination treatment explains the observed performance and carcass advantages through improved nutrient absorption and reduced gut leakage. These structural benefits carry important implications for gut health, reduced pathogen translocation and sustained productivity in resource-limited semi-arid systems. The morphological improvements agree with Saleh *et al*. [13] who documented enhanced villus architecture with betaine under heat stress. The results also corroborate Olayiwola and Adedokun[4] who linked multi-supplement strategies to greater villus height and villus to crypt ratios in tropical broiler production.

Table 7 Effect of dietary treatments on intestinal morphology (jejunum) at day 42 in arbor acres broilers under semi-arid heat stress

Parameter	Control	Vitamin C (250 mg/kg)	Betaine (2 g/kg)	Organic Se (0.3 mg/kg)	Combination	SEM	P-value
Villus height (µm)	895^{c}	1025^{b}	1080^{b}	1050^{b}	1165^{a}	52.3	<0.001
Crypt depth (µm)	185^{a}	172^{ab}	168^{b}	170^{b}	158^{b}	8.1	<0.05
Villus:crypt ratio	4.84^{c}	5.96^{b}	6.43^{b}	6.18^{b}	7.37^{a}	0.32	<0.001

^{abc} means on the same rows with different superscripts are significantly (P>0.05) different; SEM, Standard Error of Mean; P-value, Probability Value.

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Lastly, this study is novel in demonstrating the synergistic benefits of combining vitamin C (250 mg/kg), betaine (2 g/kg), and organic selenium (0.3 mg/kg as hydroxy-selenomethionine) specifically in Arbor Acres broilers under natural semi-arid heat stress conditions in northern Nigeria. While previous works have examined individual or partial combinations of these additives, the present investigation provides the first comprehensive evaluation of the full triple combination on physiological stress markers, antioxidant status, growth performance, carcass traits, and intestinal morphology simultaneously in this commercial strain and environment. The magnitude of improvement observed with the combination (particularly in rectal temperature reduction, antioxidant enzyme activities, and jejunal villus height) exceeded that reported in many single- or dual-supplement studies, confirming additive-to-synergistic interactions under prolonged natural heat load typical of the Nigerian savannah.

Conclusion

The present study demonstrates that dietary supplementation with vitamin C at 250 mg/kg, betaine at 2 g/kg, organic selenium at 0.3 mg/kg and particularly their combination effectively mitigates the adverse physiological, biochemical, performance, carcass and intestinal effects of natural semi-arid heat stress in Arbor Acres broiler chickens. The combination treatment consistently produced the most pronounced improvements, including the lowest rectal temperature, heterophil to lymphocyte ratio, serum corticosterone and malondialdehyde concentrations, alongside the highest superoxide dismutase and glutathione peroxidase activities, final body weight, feed conversion efficiency, dressing percentage, breast yield and jejunal villus height and villus to crypt ratio. These outcomes indicate synergistic interactions among the supplements that collectively enhance antioxidant defence, reduce stress hormone secretion, preserve cellular and gut integrity, optimise nutrient utilisation and support superior growth and carcass quality under prolonged high ambient temperatures typical of northern Nigeria. The findings confirm that targeted nutritional modulation represents a practical, cost-effective strategy to sustain broiler productivity and welfare in resource-constrained semi-arid environments where environmental cooling infrastructure remains limited. Therefore, the combination of vitamin C (250 mg/kg), betaine (2 g/kg) and organic selenium (0.3 mg/kg) in broiler diets is recommended for commercial production in semi-arid regions to alleviate heat stress and maximise economic returns.

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None.

Conflicts of interest

The Authors declares that there are no conflicts of interest.

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