



Original Research Paper

Effects of Graded Levels of Ethanolic Romanesco Cauliflower (*Brassica oleracea* var. *botrytis*) Leaf Extract in Drinking Water on Growth Performance and Functional and Sensory Meat Traits of Ross 308 Broilers under Cyclic Heat Stress

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Abstract

The aim of this experiment was to determine the effect of ethanolic Romanesco cauliflower (*Brassica oleracea* var. *botrytis*) leaf extract when supplemented in drinking water on the negative effects of cyclic heat stress in Ross 308 broilers. The 240 one-day-old chicks were assigned to 4 treatments (0, 200, 400, 600 mg dry matter/L of drinking water) and five replicates of 12 chicks were used. The trial lasted 35 days. Birds were exposed to cyclic heat stress at $34 \pm 1^\circ\text{C}$ for 8 h/day for 13 consecutive days (days 22–34 of age), before sampling at day 35. The best results were obtained at 400 mg/L, at which final body weight increased from 2084 g to 2242 g, body weight gain increased from 2039 g to 2198 g, and feed conversion ratio improved from 1.66 to 1.56, while mortality decreased from 5.00 to 1.67%. The same treatment improved dressing percentage and breast yield, decreased cooking loss, shear force, and breast-meat lipid oxidation during frozen storage for 3 months at -18°C , and increased sensory acceptability. Romanesco extract further improved antioxidant status by enhancing antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase) and antioxidant metabolites (reduced glutathione and ferric reducing antioxidant power), while decreasing serum malondialdehyde, triglycerides, cholesterol, and liver- and kidney-function indices. The results suggest that ethanolic Romanesco leaf extract can be a useful phytogetic supplement for broilers exposed to cyclic heat stress by supporting growth performance, oxidative stability, and meat quality.

Keywords: broiler Ross 308, Romanesco cauliflower -leaf extract, heat stress, phytogetic additive, antioxidant status, meat quality, sensory evaluation.

1. Introduction

Modern broiler production must sustain rapid growth while maintaining physiological stability and acceptable product quality. This challenge has increased interest in natural feed and water additives as alternatives to synthetic growth promoters. Phytogetic materials can provide phenolics, flavonoids, carotenoids, vitamins, and other metabolites that may support antioxidant

capacity, nutrient utilization, and postmortem meat stability (Aviagen, 2025; Oke et al., 2024; Oni et al., 2024). Nevertheless, the efficacy of phytogetic products cannot be generalized across plant species, extraction procedures, doses, or routes of administration; each preparation requires direct evaluation under a defined environmental challenge.

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Heat stress is among the most damaging environmental constraints in broiler production. Reduced feed intake explains only part of the associated performance loss, because endocrine disruption, oxidative injury, altered nutrient partitioning, and impaired muscle metabolism also contribute to poorer growth, carcass yield, and meat quality (Lin et al., 2006; Oke et al., 2024; Prates, 2025; Saad et al., 2023). Although several nutritional strategies have improved selected responses under heat stress, differences in thermal protocols and additive composition make comparisons difficult, and improvements in live performance do not necessarily translate into better oxidative stability or sensory quality of stored meat.

Romanesco cauliflower leaves are an underused by-product of *Brassica oleracea* var. botrytis and contain phenolic acids, flavonoids, glucosinolates, carotenoids, and vitamin C (Drabińska et al., 2021; Kosewski et al., 2023; Shinali et al., 2024). Previous studies provide useful but incomplete evidence. Hu et al. (2012) showed that broccoli stem-and-leaf meal affected broiler antioxidant function and meat quality, but the material was provided as a dietary meal rather than as a standardized ethanolic extract in drinking water. Mahesh et al. (2026) evaluated cauliflower waste in broilers and focused primarily on thyroid, antioxidant, immune, and molecular responses, without integrating frozen-storage lipid oxidation and sensory outcomes. Thus, evidence from related *Brassica* materials supports biological plausibility but does not establish the effective dose, delivery route, or postmortem benefits of Romanesco leaf extract under cyclic heat stress.

The research gap is therefore the absence of a dose-response study evaluating ethanolic Romanesco cauliflower leaf extract administered through drinking water while simultaneously measuring live performance, systemic oxidative and biochemical responses, carcass and fresh-meat traits, lipid oxidation during frozen storage, and sensory quality in heat-stressed broilers. Accordingly, the objective of this study was to determine the effects of 0, 200, 400, and 600 mg dry matter/L of ethanolic Romanesco leaf extract on these outcomes in Ross 308 broilers exposed to cyclic heat stress. It was hypothesized that an intermediate concentration would provide the greatest benefit by improving antioxidant protection and metabolic adaptation without the loss of efficacy that may occur at a higher phytogenic dose.

2. Materials and Methods

2.1. Ethical approval

All bird-handling procedures were performed according to the ethical guidelines of the relevant institutional committee and according to accepted guidelines of poultry handling, thermal challenge, blood collection and slaughter.

2.2. Experimental location and birds

A total of 240 one-day-old Ross 308 broiler chicks were used in a 35-day trial. After arrival, the chicks were individually weighed, marked as required, and randomly assigned to the experimental treatments.

2.3. Experimental design and heat stress conditions

The experiment followed a completely randomized design with four treatments, five replicate floor pens per treatment, and 12 birds per pen (approximately 12 birds/m²). Feed and water were offered ad libitum. All groups received the same sanitation, vaccination, and general management; treatments differed only in the concentration of Romanesco extract supplied in drinking water. Cyclic heat stress was imposed for 13 consecutive days, from day 22 through day 34, by increasing ambient temperature to $34 \pm 1^\circ\text{C}$ for 8 h/day (09:00–17:00); the recommended age-specific temperature was maintained during the remaining 16 h. Temperature was monitored at bird level throughout the trial, and relative humidity during the heat-stress period ranged from 60 to 65%. The four treatments were T1, plain drinking water; T2, 200 mg dry matter/L; T3, 400 mg dry matter/L; and T4, 600 mg dry matter/L of ethanolic Romanesco cauliflower leaf extract. This cyclic model was selected because comparable protocols produce measurable changes in broiler performance, oxidative balance, and meat quality (Lin et al., 2006; Shakeri et al., 2019; Qader & Tayeb, 2024).

2.4. Preparation of ethanolic Romanesco cauliflower leaf extract

The ethanolic extract was prepared according to Harborne (1973). Fresh Romanesco cauliflower leaves were washed, shade-dried, and ground to a fine powder. A 100-g portion of the dried leaf powder was mixed thoroughly with 500 mL of 98% ethanol (1:5, w/v) and macerated for 24 h at room temperature (25°C). The mixture was filtered once through Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure at 40°C using a rotary vacuum evaporator to remove the ethanol and was then held at room temperature until the residual solvent had evaporated.

completely and a highly viscous crude extract was obtained. A stock solution was prepared by dispersing 2 g of the concentrated extract in 50 mL of distilled water, giving an extract concentration of 40 mg/mL. The required volume of this stock was freshly diluted in drinking water each day to provide 200, 400, or 600 mg extract dry matter/L.

The phytochemical composition of the ethanolic Romanesco cauliflower leaf extract was determined by high-performance liquid chromatography (HPLC) following a validated protocol for plant extracts. Compounds were identified by comparison with authentic reference standards and quantified by external-standard calibration. The following constituents were detected (mg/g extract dry weight): hesperidin, 148.75; catechin, 51.60; naringin, 34.85; apigenin-6-O-arabinose-8-O-galactose, 29.40; pyrogallol, 27.90; luteolin-7-O-glucoside, 19.75; and benzoic acid, 18.30.

Volume of stock solution added (mL) = [required extract concentration (mg/L) × volume of drinking water (L)] / 40 mg/mL.

2.5. Basal diets and feeding program

All birds received the same basal diets, and Romanesco extract was supplied only through drinking water. The ingredient inclusion levels and calculated nutrient contents of the starter (0–10 days), grower (11–24 days), and finisher (25–35 days) diets are presented in Table 1. Diets were formulated to meet the age-specific recommendations for Ross 308 broilers (Aviagen, 2022, 2025).

2.6. Growth performance measurements

Live body weight, weight gain, feed consumption, feed conversion ratio and mortality percentage were measured weekly as indicators of growth performance. The body weight gain was computed as the difference in body weight at the end of each period and the beginning body weight for each period, and the feed conversion ratio was calculated as feed intake divided by the body weight gain for each period.

2.7. Slaughter procedure and carcass evaluation

At 35 days of age, nine birds were randomly selected from each replicate pen after an appropriate feed-withdrawal period, with free access to water. The selected birds were slaughtered following standard poultry procedures. For the frozen-storage study, three birds from each of the five replicate pens were randomly allocated to each of the three storage durations (0, 45, or 90

days), providing 15 independent birds per treatment × storage-duration combination. Each bird contributed breast-meat samples to only one storage duration. Carcass yield and the relative weights of the breast, thighs, and wings were subsequently recorded and expressed as percentages of the corresponding live body weight or carcass weight, as appropriate.

2.8. Functional meat quality traits

Breast-muscle samples were utilized to measure pH, cooking loss, Warner-Bratzler shear force and instrument color coordinates (L^* , a^* , and b^*). The pH of the muscle was determined using a calibrated portable pH meter, the cooking loss was determined as the percentage of weight lost during cooking, the tenderness was determined by the shear force, and the color was determined on the surface of the fillets by a colorimeter as per the standard procedure for poultry meat quality (Sujiwo et al., 2018; Prates, 2025).

2.9. Lipid oxidation during frozen storage

Breast-meat samples were collected from different birds and assigned independently to frozen-storage durations of 0, 45, or 90 days at -18°C . Fifteen independent breast-meat samples were allocated to each treatment × storage-duration combination ($n = 15$), yielding a total of 180 observations. Each bird contributed one sample to only one storage duration; therefore, the observations were considered independent. At the end of each designated storage period, thiobarbituric acid reactive substances (TBARS) were determined and expressed as malondialdehyde (MDA; mg MDA/kg meat) to assess the progression of lipid oxidation during frozen storage (Sujiwo et al., 2018; Varzaru et al., 2024).

2.10. Sensory evaluation

Sensory evaluation was conducted only after 90 days of frozen storage. After skinning, the Pectoralis major muscles were chilled at 4°C for 24 h, packaged, and stored at -18°C for 90 days. At the end of the storage period, the samples were thawed at 4°C for 24 h, cut into uniform portions of approximately 100 g, and cooked on an electric grill at 180°C until an internal temperature of 75°C was reached. The samples were served warm (approximately 60°C) on white plates labeled with randomized three-digit codes. Evaluation was conducted in a dedicated sensory room under red lighting at $22 \pm 2^{\circ}\text{C}$ and 50–60% relative humidity. The panel comprised 10 trained assessors (five men and five women, aged 25–45 years), who completed three preliminary training sessions of 90 min each using reference samples for the attributes evaluated. Flavor, tenderness, juiciness, and overall acceptability were assessed using a 9-point hedonic

scale (1 = dislike extremely; 9 = like extremely). Samples were presented in random order during separate sessions to minimize sensory fatigue, and water and unsalted crackers were provided between samples as palate cleansers. Panel training, test-room control, randomized sample presentation, and hedonic assessment followed established sensory-evaluation principles (Civille et al., 2025).

2.11. Blood biochemical and oxidative stress parameters

Blood samples were collected from slaughtered birds at 35 days of age, and serum was separated by centrifugation. All serum biochemical, oxidative-stress, and thyroid-hormone measurements were performed in an external laboratory using commercial assay kits in accordance with the manufacturers' instructions. The measured variables comprised antioxidant biomarkers (SOD, GPx, catalase, GSH, MDA, and FRAP), thyroid hormones (T3 and T4), liver- and kidney-function indices (ALT, AST, creatinine, and urea-N), and lipid and protein traits (triglycerides, cholesterol, albumin, and total protein). These variables were selected to characterize oxidative status (Oke et al., 2024), endocrine adjustment (Mahesh et al., 2026), and metabolic health (Mohsin et al., 2025) during nutritional and thermal challenge.

2.12. Statistical analysis

Data for growth performance, carcass traits, fresh-meat quality, sensory traits, and blood variables were analyzed by one-way analysis of variance according to Gomez and Gomez (1984), with supplementation treatment as the fixed effect. Replicate pen was the experimental unit for growth-performance traits; the sampling unit nested within pen was used for carcass, meat-quality, sensory, and blood traits. Breast-meat MDA was analyzed by two-factor analysis of variance, with supplementation level (T), frozen-storage duration (S), and the $T \times S$ interaction included as fixed effects. The model was $Y_{ijk} = \mu + T_i + S_j + (T \times S)_{ij} + e_{ijk}$.

When a fixed effect or interaction was significant, means were separated using Duncan's multiple range test at $P \leq 0.05$. Mortality percentages were arcsine-transformed when necessary to satisfy model assumptions. Table 4 reports the analysis-of-variance components, interaction-cell means, marginal means for supplementation and storage duration, and the treatment comparison within each storage duration.

3. Results

3.1 Productive performance

Romanesco leaf extract affected final body weight, body weight gain, feed conversion ratio, and mortality, whereas feed intake did not differ among treatments (Table 2). The response was dose-dependent up to 400 mg/L and then plateaued at 600 mg/L.

Relative to the unsupplemented control, 400 mg/L increased final body weight by 158 g and body weight gain by 159 g, improved feed conversion ratio from 1.66 to 1.56, and reduced mortality from 5.00% to 1.67% ($P \leq 0.05$). Because feed intake was unchanged ($P = 0.412$), the growth response reflected improved efficiency rather than greater feed consumption.

3.2 Carcass traits and meat quality

Supplementation improved dressing percentage, breast yield, pH at 24 h, cooking loss, shear force, and meat color, whereas thigh and wing yields were unaffected (Table 3). The most favorable values generally occurred at 400 mg/L, with no additional improvement at 600 mg/L.

Compared with the control, 400 mg/L increased dressing percentage and breast yield by 2.4 percentage points each, reduced cooking loss by 3.6 percentage points, and decreased shear force by 3.8 N ($P \leq 0.05$). The concurrent increase in pH and redness and decrease in lightness were consistent with better water retention and preservation of muscle structure.

3.3 Lipid oxidation during frozen storage

Two-factor analysis of breast-meat MDA showed significant effects of supplementation, frozen-storage duration, and their interaction ($P < 0.001$; Table 4). The storage marginal mean increased from 0.31 mg/kg at day 0 to 0.60 mg/kg at day 45 and 0.97 mg/kg at day 90, whereas the supplementation marginal mean was lowest at 400 mg/L (0.51 mg/kg) compared with the control (0.81 mg/kg).

The significant supplementation $\times$ storage-duration interaction reflected the progressive separation among treatments during storage. At day 0, MDA did not differ among treatments ($P = 0.084$). Differences emerged after 45 days ($P = 0.002$) and were greatest after 90 days, when MDA was 1.31

mg/kg in the control and 0.76 mg/kg at 400 mg/L ($P < 0.001$), a reduction of approximately 42%. The 600 mg/L treatment did not improve oxidative stability beyond the 400 mg/L treatment.

3.4 Sensory evaluation

Sensory scores supported the instrumental meat-quality findings (Table 5). Flavor, tenderness, juiciness, and overall acceptability were higher at 400 mg/L than in the control ($P \leq 0.05$), whereas 600 mg/L produced no further improvement.

3.5 Oxidative stress biomarkers and thyroid hormones

Romanesco supplementation improved systemic antioxidant status and thyroid-hormone concentrations (Table 6). At 400 mg/L, SOD, GPx, catalase, GSH, and FRAP were higher and serum MDA was lower than in the control ($P < 0.001$). T3 and T4 also increased ($P = 0.033$ and $P = 0.028$, respectively), indicating a more favorable redox state and metabolic adjustment during cyclic heat stress.

3.6 Blood biochemical profile

Romanesco supplementation also altered the serum biochemical profile (Table 7). At 400 mg/L, ALT, AST, creatinine, urea-N, triglycerides, and cholesterol were lower, whereas albumin and total protein were higher than in the control ($P \leq 0.05$). The 600 mg/L treatment was statistically comparable with 400 mg/L for these variables, confirming that the response had plateaued.

4. Discussion

The present results show that ethanolic Romanesco cauliflower leaf extract improved growth efficiency in broilers exposed to cyclic heat stress, with the most consistent response at 400 mg/L. The higher body-weight gain without increased feed intake indicates more efficient nutrient utilization rather than an appetite-mediated effect. Heat stress increases maintenance requirements, disrupts thyroid and metabolic signaling, and diverts nutrients from growth toward thermoregulation and cellular defense (Oke et al., 2024; Prates, 2025). The simultaneous improvement in feed conversion, circulating T3 and T4, antioxidant indices, and

serum biochemical traits therefore supports a coordinated metabolic and redox response rather than an isolated change in growth.

The chemical profile of the experimental extract provides a plausible phytochemical basis for the coordinated physiological response. Hesperidin was the predominant quantified constituent (148.75 mg/g dry weight), followed by catechin (51.60 mg/g), naringin (34.85 mg/g), apigenin-6-O-arabinose-8-O-galactose (29.40 mg/g), pyrogallol (27.90 mg/g), luteolin-7-O-glucoside (19.75 mg/g), and benzoic acid (18.30 mg/g). These phenolic and flavonoid constituents may contribute to radical scavenging, endogenous antioxidant defense, and reduced membrane lipid peroxidation, consistent with the increased SOD, GPx, catalase, GSH, and FRAP and the lower MDA observed at 400 mg/L. Nevertheless, the contribution of each constituent cannot be separated from the combined action of the crude extract.

The findings also extend the limited poultry literature on Brassica by-products. Hu et al. (2012) reported that broccoli stem-and-leaf meal enhanced antioxidant function, skin pigmentation, and aspects of meat quality in broilers, supporting the capacity of cruciferous leaf material to influence systemic redox balance and muscle traits. Mahesh et al. (2026) likewise found that cauliflower waste affected thyroid hormones, antioxidant status, immunity, and stress-related gene expression. In contrast to those studies, the present work used an ethanolic Romanesco leaf extract delivered in drinking water and linked live-bird responses with carcass yield, fresh-meat properties, lipid oxidation over 90 days of frozen storage, and sensory quality. These differences in plant material, extraction, delivery route, and outcomes limit direct numerical comparison but strengthen the evidence that Brassica by-products can act across both physiological and product-quality domains.

The plateau beyond 400 mg/L is consistent with a nonlinear phytogenic dose response. At the intermediate concentration, antioxidant and metabolic pathways may have approached their effective response ceiling, as indicated by the absence of additional improvements in SOD, GPx, catalase, GSH, FRAP, thyroid hormones, carcass yield, or meat quality at 600 mg/L. Increasing the crude extract above this point would not necessarily increase biological availability because absorption, biotransformation, and cellular antioxidant recycling are saturable. Moreover, a

higher total exposure to the quantified phenolic and flavonoid constituents could introduce competing or antagonistic effects that offset further benefit. Although seven constituents were quantified, their absorption, metabolism, and individual contribution to the response were not measured; therefore, physiological saturation and phytochemical interaction remain mechanistic hypotheses requiring targeted dose-response studies.

Improved dressing percentage and breast yield suggest that the extract helped preserve lean-tissue deposition during heat stress. The lower cooking loss and shear force, together with the changes in pH and color, indicate better maintenance of muscle membrane integrity and postmortem water-holding capacity. Thermal stress accelerates glycogen depletion and oxidative modification of membrane lipids and proteins, processes that impair water retention, tenderness, and appearance. The current response is therefore consistent with the protection of muscle structure by nutritional antioxidants under cyclic heat challenge (Shakeri et al., 2019; Pečjak Pal et al., 2024; Prates, 2025), and it is directly comparable in direction with the meat-quality effects reported for broccoli stem-and-leaf meal by Hu et al. (2012).

The storage and sensory findings show that the benefit persisted after slaughter. MDA rose with storage time in every group, as expected from progressive lipid oxidation, but the significant supplementation × storage-duration interaction demonstrated that this increase was attenuated by Romanesco supplementation. At 400 mg/L, the approximately 42% lower MDA after 90 days at -18°C coincided with higher flavor, tenderness, juiciness, and overall acceptability. This agreement between chemical, instrumental, and sensory outcomes suggests that greater *in vivo* antioxidant protection carried over into the muscle. Similar links between nutritional antioxidants, oxidative stability, and poultry-meat quality have been described under heat stress and during storage (Shakeri et al., 2019; Pečjak Pal et al., 2024; Varzaru et al., 2024).

5. Conclusions

Ethanollic Romanesco cauliflower leaf extract supplementation in drinking water improved growth performance, antioxidant status, carcass yield, frozen-meat stability, sensory quality, and selected biochemical characteristics in Ross 308

broilers during cyclic heat stress. Across the measured responses, 400 mg/L provided the most balanced and biologically useful response, whereas increasing the concentration to 600 mg/L produced no additional benefit. These findings support the use of Romanesco leaves as a functional Brassica by-product in broiler production under hot conditions. The study was limited to a single cyclic heat-stress protocol, and the chemical profile was restricted to seven quantified constituents; confirmation under commercial conditions and broader compositional characterization are therefore warranted.

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References

- Aviagen. (2022). Ross Broiler: Nutrition Specifications. Aviagen.
https://aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerNutritionSpecifications2022-EN.pdf
- Aviagen. (2025). Ross Broiler Nutrition Supplement. Aviagen.
https://aviagen.com/assets/Tech_Center/Ross_Broiler/Aviagen_Ross_BroilerNutritionSupplement.pdf
- Civille, G. V., Carr, B. T., & Osdoba, K. E. (2025). Sensory Evaluation Techniques (6th ed.). CRC Press.
- Drabińska, N., Jež, M., & Nogueira, M. (2021). Variation in the accumulation of phytochemicals and their bioactive properties among the aerial parts of cauliflower. *Antioxidants*, 10(10), 1597.
<https://doi.org/10.3390/antiox10101597>
- Gomez, K. A., & Gomez, A. A. (1984). Statistical Procedures for Agricultural Research (2nd ed.). John Wiley & Sons.
- Harborne, J. B. (1973). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Chapman and Hall.
- Hu, C. H., Wang, D. G., Pan, H. Y., Zheng, W. B., Zuo, A. Y., & Liu, J. X. (2012). Effects of broccoli stem and leaf meal on broiler performance, skin pigmentation,

antioxidant function, and meat quality. *Poultry Science*, 91(11), 2858-2864. <https://doi.org/10.3382/ps.2012-02142>

Kosewski, G., Kowalówka, M., Drzymała-Czyż, S., & Przysławski, J. (2023). The impact of culinary processing, including sous-vide, on polyphenols, vitamin C content and antioxidant status in selected vegetables—methods and results: A critical review. *Foods*, 12(11), 2121. <https://doi.org/10.3390/foods12112121>

Lin, H., Jiao, H. C., Buyse, J., & Decuypere, E. (2006). Strategies for preventing heat stress in poultry. *World's Poultry Science Journal*, 62(1), 71-86. <https://doi.org/10.1079/WPS200585>

Mahesh, T., Biswas, A., & Khandare, R. (2026). Exploring nutritional evaluation of cauliflower (*Brassica oleracea* var. botrytis) waste on the thyroid hormone, antioxidant status, immunity and expression of Interleukin-6 and HSP70 genes in broilers. *Waste and Biomass Valorization*. <https://doi.org/10.1007/s12649-026-03499-x>

Mohsin, R. H., Al-Hummod, S. K., & Saad, H. F. (2025). Effect of quinoa seed powder and alcoholic extract on productive performance and some biological and physiological blood parameters of broilers exposed to heat stress. *NEsciences*, 10(3), 1-15.

Oke, O. E., Gomes, A. V. S., Madeira, M. S., Rocha, C., & Prates, J. A. M. (2024). Oxidative stress in poultry production. *World's Poultry Science Journal*, 80(4), 1117-1141. <https://doi.org/10.1080/00439339.2024.2411963>

Oni, A. I., Adeleye, O. O., Adebawale, T. O., & Oke, O. E. (2024). The role of phytogenic feed additives in stress mitigation in broiler chickens. *Journal of Animal Physiology and Animal Nutrition*, 108(1), 81-98. <https://doi.org/10.1111/jpn.13869>

Pečjak Pal, M., Leskovec, J., Levart, A., Pirman, T., Salobir, J., & Rezar, V. (2024). Comparison of high n-3 PUFA levels and cyclic heat stress effects on carcass characteristics, meat quality, and oxidative status in

broilers. *Poultry Science*, 103, 103554. <https://doi.org/10.1016/j.psj.2024.103554>

Prates, J. A. M. (2025). Impact of heat stress on carcass traits, meat quality, and nutritional value in monogastric animals: Underlying mechanisms and nutritional mitigation strategies. *Animals*, 15(2), 180. <https://doi.org/10.3390/ani15020180>

Qader, G. K., & Tayeb, I. T. (2024). Influence of medicinal plants and vitamin E on productive performance and some physiological parameters of broiler chickens under heat stress. *Iraqi Journal of Agricultural Sciences*, 55(2), 790-801.

Saad, H. F., Alsudany, S. M., Al-Hummod, S. K. M., & Jaffar, A. A. (2023). The impact of high temperatures on the productive performance (behavioral, physiological, and immunological) of poultry. *Texas Journal of Agriculture and Biological Sciences*, 14, 52-57.

Shakeri, M., Cottrell, J. J., Wilkinson, S., Le, H. H., Suleria, H. A. R., Warner, R. D., & Dunshea, F. R. (2019). Growth performance and characterization of meat quality of broiler chickens supplemented with betaine and antioxidants under cyclic heat stress. *Antioxidants*, 8(9), 336. <https://doi.org/10.3390/antiox8090336>

Shinali, T. S., Zhang, Y., Altaf, M., Nsabiyeze, A., Han, Z., Shi, S., & Shang, N. (2024). The valorization of wastes and byproducts from cruciferous vegetables: A review on the potential utilization of cabbage, cauliflower, and broccoli byproducts. *Foods*, 13(8), 1163. <https://doi.org/10.3390/foods13081163>

Sujiwo, J., Kim, D., & Jang, A. (2018). Relation among quality traits of chicken breast meat during cold storage: Correlations between freshness traits and torrymeter values. *Poultry Science*, 97(8), 2887-2894. <https://doi.org/10.3382/ps/pey138>

Varzaru, I., Panaite, T. D., Turcu, R. P., Untea, A. E., Ropota, M., Omrani, S. M., & Vlaicu, P. A. (2024). *Chlorella vulgaris* as a nutraceutical source for broilers: Improving meat quality, oxidative stability, and nutritional value. *Animals*, 14(14), 2124. <https://doi.org/10.3390/ani14142124>

Table 1. Ingredient composition and calculated nutrient content of the basal diets used during the starter, grower, and finisher phases

Element / Parameter	Starter (0–10 Days)	Grower (11–24 Days)	Finisher (25+ Days)
Ingredient Inclusion (%)			
Yellow corn	53.50	58.20	63.40
Soybean meal (44% CP)	38.50	33.20	28.10
Soybean oil	2.50	3.50	4.00
Limestone	1.25	1.15	1.05
Dicalcium phosphate (DCP)	1.85	1.65	1.40
Salt (NaCl)	0.35	0.35	0.35
Vitamin & Mineral Premix	0.50	0.50	0.50
DL-Methionine	0.38	0.32	0.28
L-Lysine HCl	0.17	0.18	0.22
Inert filler (Sand)*	1.00	0.95	0.70
Total	100.00	100.00	100.00
Calculated Nutrient Content			
Metabolizable Energy (kcal/kg)	3,000	3,100	3,200
Crude Protein (%)	23.00	21.00	19.00
Crude Fiber (%)	3.60	3.40	3.20
Ether Extract (%)	4.80	5.90	6.50
Calcium (%)	0.96	0.87	0.78
Available Phosphorus (%)	0.48	0.44	0.39
Digestible Lysine (%)	1.28	1.15	1.02
Digestible Methionine (%)	0.62	0.54	0.48

CP = crude protein; DCP = dicalcium phosphate. * An inert filler (sand) was added to round the total mixture of ingredients to exactly 100.00%.

Table 2. Effect of Romanesco cauliflower leaf extract on overall productive performance of Ross 308 broilers reared under heat stress conditions (1–35 d)

Trait	T1 Control	T2 200 mg/L	T3 400 mg/L	T4 600 mg/L	SE M	P-value
Final body weight (g)	2084 ^b	2161 ^{ab}	2242 ^a	2218 ^a	22.4	0.013
Body weight gain (g)	2039 ^b	2117 ^{ab}	2198 ^a	2173 ^a	22.7	0.015
Feed intake (g/bird)	3384	3410	3421	3405	18.1	0.412
FCR	1.66 ^a	1.61 ^a _b	1.56 ^b	1.57 ^b	0.02	0.021
Mortality (%)	5.00 ^a	3.33 ^a _b	1.67 ^b	1.67 ^b	0.61	0.039

Values are presented as means $\pm$ SEM. Means within the same row bearing different superscript letters (a–c) differ significantly at $P \leq 0.05$. T1 = control group (plain drinking water without additive); T2 = ethanolic Romanesco cauliflower leaf extract at 200 mg dry matter/L drinking water; T3 = ethanolic Romanesco cauliflower leaf extract at 400 mg dry matter/L drinking water; T4 = ethanolic Romanesco cauliflower leaf extract at 600 mg dry matter/L drinking water. SEM = standard error of the mean; FCR = feed conversion ratio.

Table 3. Effect of Romanesco cauliflower leaf extract on carcass traits and fresh meat quality of Ross 308 broilers reared under heat stress conditions at day 35

Trait	T1 Control	T2 200 mg/L	T3 400 mg/L	T4 600 mg/L	SE M	P-value
Dressing percentage (%)	71.8 ^b	73.0 ^{ab}	74.2 ^a	73.8 ^a	0.44	0.018
Breast yield (%)	30.4 ^b	31.5 ^{ab}	32.8 ^a	32.4 ^a	0.33	0.011
Thigh yield (%)	21.9	22.1	22.4	22.3	0.21	0.247
Wing yield (%)	9.4	9.5	9.6	9.6	0.09	0.531
pH (24 h)	5.86 ^b	5.93 ^{ab}	6.00 ^a	5.98 ^a	0.03	0.026
Cooking loss (%)	25.4 ^a	23.7 ^b	21.8 ^c	22.2 ^{bc}	0.54	0.004
Shear force (N)	21.7 ^a	19.8 ^b	17.9 ^c	18.4 ^{bc}	0.62	0.003
L*	56.3 ^a	55.2 ^{ab}	54.0 ^b	54.4 ^b	0.49	0.031
a*	2.8 ^b	3.3 ^{ab}	3.9 ^a	3.8 ^a	0.17	0.012
b*	9.8 ^b	10.2 ^{ab}	10.7 ^a	10.5 ^a	0.19	0.041

Values are presented as means $\pm$ SEM. Means within the same row bearing different superscript letters (a–c) differ significantly at $P \leq 0.05$. T1 = control group (plain drinking water without additive); T2 = ethanolic Romanesco cauliflower leaf extract at 200 mg dry matter/L drinking water; T3 = ethanolic Romanesco cauliflower leaf extract at 400 mg dry matter/L drinking water; T4 = ethanolic Romanesco cauliflower leaf extract at 600 mg dry matter/L drinking water. L* = lightness; a* = redness; b* = yellowness; SEM = standard error of the mean.

Table 4. Factorial effects of Romanesco cauliflower leaf extract supplementation and frozen-storage duration on breast-meat MDA concentration (mg MDA/kg meat) in Ross 308 broilers under cyclic heat stress

A. Analysis of variance						
Source of variation	df	Mean square	F-value	P-value		
Supplementation (T)	3	0.184	48.7	<0.001		
Storage duration (S)	2	1.872	495.2	<0.001		
T × S interaction	6	0.041	10.9	<0.001		
Error	168	0.0038	—	—		
B. Interaction means						
Storage duration	T1 Control	T2 200 mg/L	T3 400 mg/L	T4 600 mg/L	Storage mean	P (treatment)
Day 0	0.34	0.31	0.28	0.29	0.31	0.084
Day 45	0.78 ^a	0.62 ^b	0.49 ^c	0.52 ^c	0.60	0.002
Day 90	1.31 ^a	1.01 ^b	0.76 ^c	0.81 ^c	0.97	<0.001
Supplementation mean	0.81	0.65	0.51	0.54	0.63	—

Within a storage duration, means bearing different superscript letters (a–c) differ at $P \leq 0.05$. P (treatment) refers to the treatment comparison within each duration. T1 = control; T2 = 200; T3 = 400; and T4 = 600 mg extract dry matter/L. MDA = malondialdehyde. n = 15 independent samples per treatment × storage-duration combination.

Table 5. Sensory scores of cooked breast meat from Ross 308 broilers reared under heat stress conditions after 3 months of frozen storage at -18°C

Trait (9-point scale)	T1 Control	T2 200 mg/L	T3 400 mg/L	T4 600 mg/L	SEM	P-value
Flavor	6.1 ^b	6.8 ^{ab}	7.5 ^a	7.3 ^a	0.19	0.017
Tenderness	6.0 ^b	6.9 ^{ab}	7.6 ^a	7.4 ^a	0.18	0.009
Juiciness	5.9 ^b	6.7 ^{ab}	7.4 ^a	7.2 ^a	0.21	0.015
Overall acceptability	6.0 ^b	6.9 ^{ab}	7.7 ^a	7.4 ^a	0.20	0.008

Values are presented as means ± SEM. Means within the same row bearing different superscript letters (a–c) differ

significantly at $P \leq 0.05$. T1 = control group (plain drinking water without additive); T2 = ethanolic Romanesco cauliflower leaf extract at 200 mg dry matter/L drinking water; T3 = ethanolic Romanesco cauliflower leaf extract at 400 mg dry matter/L drinking water; T4 = ethanolic Romanesco cauliflower leaf extract at 600 mg dry matter/L drinking water.

Table 6. Effect of Romanesco cauliflower leaf extract on serum antioxidant indices and thyroid hormones of Ross 308 broilers reared under heat stress conditions at day 35

Trait	T1 Control	T2 200 mg/L	T3 400 mg/L	T4 600 mg/L	SEM	P-value
SOD (U/mL)	82.4 ^c	90.6 ^b	98.9 ^a	96.8 ^a	1.81	<0.001
GPx (U/L)	71.8 ^c	78.6 ^b	86.2 ^a	84.9 ^a	1.72	<0.001
Catalase (U/L)	7.1 ^c	7.9 ^b	8.8 ^a	8.6 ^a	0.16	<0.001
GSH (μmol/L)	2.92 ^c	3.31 ^b	3.81 ^a	3.72 ^a	0.08	<0.001
MDA (μmol/L)	5.23 ^a	4.45 ^b	3.77 ^c	3.95 ^c	0.12	<0.001
FRAP (mmol Fe ²⁺ /L)	0.88 ^c	0.99 ^b	1.12 ^a	1.08 ^a	0.03	<0.001
T3 (ng/mL)	1.61 ^b	1.70 ^{ab}	1.82 ^a	1.79 ^a	0.04	0.033
T4 (μg/dL)	9.1 ^b	9.7 ^{ab}	10.4 ^a	10.2 ^a	0.23	0.028

Values are presented as means ± SEM. Means within the same row bearing different superscript letters (a–c) differ significantly at $P \leq 0.05$. T1 = control group (plain drinking water without additive); T2 = ethanolic Romanesco cauliflower leaf extract at 200 mg dry matter/L drinking water; T3 = ethanolic Romanesco cauliflower leaf extract at 400 mg dry matter/L drinking water; T4 = ethanolic Romanesco cauliflower leaf extract at 600 mg dry matter/L drinking water. SOD = superoxide dismutase; GPx = glutathione peroxidase; GSH = reduced glutathione; MDA = malondialdehyde; FRAP = ferric reducing antioxidant power; T3 = triiodothyronine; T4 = thyroxine; SEM = standard error of the mean.

Table 4. Main and interactive effects of Romanesco cauliflower leaf extract supplementation and frozen-storage duration on breast-meat (MDA) concentration (mg MDA/kg meat) in Ross 308 broilers exposed to cyclic heat stress.

Trait	T1 Contr	T2 200	T3 400	T4 600	SE M	P-value
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	ol	mg/ L	mg/ L	mg/ L		e
ALT (U/L)	23.2 ^a	21.0 _{ab}	18.4 _b	18.8 _b	0.87	0.01 ₉
AST (U/L)	236 ^a	223 ^a _b	208 ^b	211 ^b	6.1	0.02 ₇
Creatinine (mg/dL)	0.46 ^a	0.42 _{ab}	0.39 _b	0.40 _b	0.01	0.04 ₁
Urea-N (mg/dL)	6.4 ^a	5.9 ^{ab}	5.4 ^b	5.5 ^b	0.18	0.03 ₆
Triglycerides (mg/dL)	112 ^a	103 ^b	95 ^c	97 ^c	2.4	0.00 ₄
Cholesterol (mg/dL)	151 ^a	142 ^b	131 ^c	134 ^c	2.8	0.00 ₃
Albumin (g/dL)	1.65 ^b	1.74 _{ab}	1.84 _a	1.82 _a	0.04	0.02 ₉
Total protein (g/dL)	3.95 ^b	4.11 _{ab}	4.32 _a	4.28 _a	0.07	0.02 ₂

Values are presented as means $\pm$ SEM. Within the same row, means bearing different superscript letters (a–c) differ significantly at $P \leq 0.05$, whereas means bearing the same superscript letter do not differ significantly. T1 = control group (plain drinking water without supplementation); T2 = ethanolic Romanesco cauliflower leaf extract at 200 mg dry matter/L drinking water; T3 = ethanolic Romanesco cauliflower leaf extract at 400 mg dry matter/L drinking water; T4 = ethanolic Romanesco cauliflower leaf extract at 600 mg dry matter/L drinking water. ALT = alanine aminotransferase; AST = aspartate aminotransferase; urea-N = urea nitrogen; SEM = standard error of the mean.