REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article Received: 26/07/2024, Accepted: 08/11/2024, Published: 19/12/2024

Assessment of combined effects of thermal acclimation, probiotics, and *Bunium bulbocastanum* essential oil on performance, carcass traits, and meat quality in heat-stressed broilers

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Abstract
Heat stress poses a significant challenge to poultry production, negatively impacting broiler growth performance, feed efficiency, and meat quality. This study investigated the synergistic effects of early-age thermotolerance acclimation, probiotics, and *Bunium bulbocastanum* (BB) essential oil supplementation on broiler performance, carcass traits, and meat quality under heat stress conditions. Eighty Arbor Acres broiler chicks were randomly assigned to four experimental groups: Control (CON), Thermotolerance Acclimation (TA), Probiotic Supplementation (PRO), and Essential Oil Supplementation (BBEO). The PRO and BBEO groups demonstrated notable improvements, with the PRO group achieving the highest final body weight (2.36 ± 0.04 kg) and an optimized feed conversion ratio (FCR, 1.55 ± 0.02). Carcass yield was significantly enhanced in the PRO group (76.2 ± 1.4%) compared to the control (72.3 ± 1.7%). Meat quality parameters improved significantly in the BBEO group, with superior water-holding capacity (WHC, 78.4 ± 1.2%), lower cooking loss (15.3 ± 0.5%), and reduced lipid oxidation (MDA, 0.45 ± 0.02 mg/kg), demonstrating its strong antioxidant potential. Nutritional analysis revealed elevated crude protein content in the BBEO (23.1 ± 0.4%) and PRO (22.8 ± 0.5%) groups, along with reduced fat content. Additionally, the BBEO group exhibited the highest polyunsaturated fatty acid (PUFA) content (24.3 ± 1.1%), enhancing the nutritional value of the meat. These findings underscore the potential of probiotics and BB essential oil supplementation to mitigate heat stress effects and improve broiler productivity and meat quality.

Key words: Heat stress, broiler performance, probiotics, *Bunium bulbocastanum* essential oil, carcass yield, meat quality.

Introduction
Thermotolerance is a critical determinant of productivity in poultry production, particularly in regions with high ambient temperatures. Heat stress adversely affects broiler chickens by disrupting physiological and metabolic functions (Bengharbi et al., 2016), resulting in diminished growth performance, feed efficiency, and meat quality (Zineb et al., 2024). Consequently, developing effective strategies to enhance thermotolerance in broilers is essential to sustain productivity and ensure meat quality under challenging environmental conditions. Among these strategies, early-age acclimation has emerged as a promising method, where chicks are exposed to controlled stressors during developmental stages. This approach has been shown to improve resilience to heat stress in later life stages (Bengharbi et al., 2016; Zineb et al., 2021). Incorporating medicinal plants into poultry diets (Abdel-Moneim et al., 2021; Oni et al., 2024) represents another innovative approach to counteract the negative impacts of heat stress while simultaneously enhancing broiler meat quality (Alam et al., 2024). Medicinal plants are abundant in bioactive compounds with antioxidants (Mahasneh et al., 2024), antimicrobial (Mahasneh et al., 2024), and anti-inflammatory properties (Pashaei et al., 2024). These compounds not only mitigate the effects of heat stress but also improve the nutritional and sensory attributes of chicken meat. For instance, linseed has demonstrated its ability to modify fatty acid profiles and reduce fat deposition in broilers exposed to heat stress (Bengharbi et al. 2021). Additionally, Region-specific medicinal plants, such as *Bunium bulbocastanum*, provide natural antioxidants and antimicrobial agents that improve meat quality and extend shelf life (Bouhalla et al., 2024). Similarly, linseed supplementation (*Linum usitatissimum*) has been shown to influence biochemical and hematological parameters in heat-stressed broiler chickens across different ages (Bengharbi et al., 2024).
Probiotics offer another dimension to improving broiler meat quality under stress conditions. Functional probiotics, such as strains of *Lactobacillus*, have shown efficacy in supporting gut health, enhancing nutrient absorption, and modulating immune responses. These benefits are particularly valuable during periods of environmental stress (Bentahar et al., 2024). Furthermore, the synergistic use of medicinal plants and probiotics presents a novel strategy to optimize broiler meat quality and sustainability in poultry production systems (Alagawany et al., 2021; Shehata et al., 2022; Aqeel et al., 2024). Insights from grazing practices, traditionally associated with ruminants, also underscore the significance of diet and environmental interactions in influencing meat quality. Studies on grazing zones and their effects on meat quality parameters in sheep demonstrate the critical role of dietary composition and environmental conditions in shaping fatty

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acid profiles and physicochemical traits (Mustapha et al., 2023). These findings emphasize the need to integrate dietary and environmental strategies in poultry systems to optimize meat quality.
This study integrates thermotolerance strategies, medicinal plant supplementation, and probiotics to address the challenges posed by heat stress in poultry production. By exploring the combined effects of these approaches, it aims to establish sustainable practices that enhance broiler resilience and improve the nutritional, sensory, and storage attributes of their meat. This integrated approach seeks to advance poultry production by ensuring producer profitability and meeting consumer demands for high-quality meat.

Materials and methods

Animals

A total of 80 one-day-old male Arbor Acres broiler chicks were obtained from a commercial hatchery. Upon arrival, chicks were individually weighed and randomly allocated into four experimental groups (20 each). Each group was further subdivided into four replicates of 5 chicks to ensure proper replication for statistical analysis. All chicks were housed under standard environmental conditions in a temperature-controlled facility, with feed and water provided *ad libitum*. The feeding program followed standard broiler nutritional requirements, with a starter, grower, and finisher diet formulated to meet NRC (1994) recommendations.

Experimental design

Control group (CON) chicks were reared under normal conditions without any specific treatment. This group served as a baseline to evaluate the effects of thermotolerance acclimation, probiotics, and essential oil supplementation on broiler performance and meat quality under heat stress conditions. In thermotolerance acclimation group (TA), early-age acclimation was performed on day 5 of age to acquire adaptive thermotolerance at adult age (Bengharbi et al. 2016; Zineb et al., 2021). The early-age thermal exposure was implemented to trigger physiological and metabolic adaptations that would enhance thermotolerance during subsequent heat stress periods.
For the probiotic supplementation group (PRO) (Bentahar et al., 2024), a commercial probiotic product rich in *Lactobacillus* strains was administered in drinking water at the manufacturer-recommended concentration. Probiotic supplementation began on day 1 and continued until the end of the experimental period on day 42. Water containers were cleaned daily to prevent contamination and ensure the effectiveness of the probiotic treatment. This treatment aimed to improve gut health, enhance nutrient absorption, and support immune function, particularly under heat stress conditions.
In the essential oil supplementation group (BBEO), *Bunium bulbocastanum* essential oil was incorporated into the broiler diet at a concentration of 0.5 g/kg of feed (Bouhalla et al., 2024). The essential oil was obtained through hydro-distillation, ensuring purity and consistency, and thoroughly mixed into the feed to achieve homogenous distribution. Essential oil supplementation began on day 1 and continued throughout the experimental period to assess its potential antioxidant and antimicrobial effects on broiler meat quality and stress mitigation.

Carcass Yield and Organ Weights

Carcass yield and organ weights were measured to determine the impact of treatments on growth and development. Birds were slaughtered following humane procedures, and carcasses were unfeathered, eviscerated, and weighed. Organ weights, including the liver, heart, and gizzard, were recorded. Carcass yield was expressed as a percentage of live body weight. This methodology followed standardized approaches used in poultry studies (Gheisari et al., 2017; Bengharbi et al., 2021; Meira et al., 2023; Iyanda et al., 2024).

Broilers' meat assessment

At the end of the experiment (day 42), a subset of birds from each replicate was randomly selected for meat quality and composition.

pH Measurement

The pH of the meat was determined 45 minutes (initial pH) and 24 hours (ultimate pH) post-slaughter using a digital pH meter. Measurements were taken in the breast (Pectoralis major) and thigh (Biceps femoris) muscles following the method described by Qiao et al. (2002) and Ogbuewu et al (2024). Proper calibration of the pH meter was ensured before analysis, and care was taken to minimize contamination and external influences on pH readings.

Water Holding Capacity (WHC)

Water holding capacity was assessed (Barbut, 2024) using the filter paper press method, as described by Hamm (1960), with modifications by Grau and Hamm (1953). Briefly, 5 g of meat samples were placed between two filter papers and compressed under a standard weight for a specific duration. The released water was quantified, and WHC was expressed as a percentage of retained water relative to the sample weight. This method is widely adopted for evaluating meat quality in poultry studies (Kavitha et al., 2016).

Cooking Loss
Cooking loss was determined by weighing 10 g of breast and thigh samples before and after cooking. Samples were cooked in sealed plastic bags in a water bath at 80°C until the internal temperature reached 75°C, as monitored with a digital thermometer. The samples were cooled to room temperature, and the weight loss was calculated as a percentage of the initial weight (Honikel, 1998; Sari et al., 2024). This method has been validated for assessing meat juiciness and cooking yield in poultry studies.

Nutritional Composition
The nutritional composition of meat was determined following the methods of AOAC (2005). Moisture content was measured by oven drying at 105°C, crude protein by the Kjeldahl method, fat content using Soxhlet extraction, and ash content by incinerating samples in a muffle furnace at 550°C. These analyses provided insights into the effects of treatments on the proximate composition of broiler meat (Jung et al., 2010).

Fatty Acid Profile
The fatty acid profile was analyzed using gas chromatography (GC), following lipid extraction using the Folch method (Folch et al., 1957). Extracted lipids were methylated to fatty acid methyl esters (FAMEs) using methanolic potassium hydroxide and analyzed by GC with a flame ionization detector (FID). Fatty acid peaks were identified by comparing retention times with known standards, and results were expressed as percentages of total fatty acids (Chen et al., 2023). This analysis method is commonly applied to assess the nutritional value of broiler meat (Bengharbi et al., 2021; Mustapha et al., 2023).

Lipid Oxidation (MDA Analysis)
Malondialdehyde (MDA) levels were measured as an indicator of lipid oxidation using the thiobarbituric acid reactive substances (TBARS) method, as described by Botsoglou et al. (1994). Briefly, meat samples were homogenized with trichloroacetic acid (TCA) and thiobarbituric acid (TBA) solutions, heated in a water bath, and the absorbance of the resultant pink chromogen was measured at 532 nm using a spectrophotometer. MDA concentrations were expressed as mg MDA/kg of meat. This method is widely used to assess oxidative stability and antioxidant effects in meat, particularly in studies involving essential oils and dietary antioxidants (Bouhalla et al., 2024).

Statistical analysis
Data were analyzed using a one-way analysis of variance (ANOVA) to compare treatment effects among the four groups. Differences between group means were evaluated using Tukey's post-hoc test, and significance was set at $P < 0.05$. Results were presented as means ± standard errors (SE) to ensure clarity and precision in reporting. This experimental design and methodology aimed to comprehensively evaluate the effects of early-age thermotolerance acclimation, probiotics, and *Bunium bulbocastanum* essential oil supplementation on broiler performance, thermotolerance, and meat quality under heat stress conditions.

Results and discussion
Growth performance of broilers
Demonstrated in Table 1, PRO group achieved the highest final body weight **(**2.36 ± 0.04 kg**,** $P < 0.01$), followed by the BBEO group **(**2.30 ± 0.03 kg**)** and the TA group **(**2.28 ± 0.05 kg**)**. The control group showed the lowest final body weight **(**2.12 ± 0.06 kg**)**.

Table 01: Effect of different treatments on broiler growth performance

Parameter	Control (CON)	TA Group	PRO Group	BBEO Group	P-value
Final Body Weight (kg)	2.12 ± 0.06^{d}	2.28 ± 0.05^{c}	2.36 ± 0.04^{a}	2.30 ± 0.03^{b}	< 0.01
Feed Intake (g/bird)	3921 ± 80^{a}	3805 ± 76^{b}	3720 ± 70^{c}	3755 ± 74^{bc}	< 0.05
FCR	1.68 ± 0.02^{a}	1.61 ± 0.02^{b}	1.55 ± 0.02^{c}	1.57 ± 0.03^{bc}	< 0.01

The feed conversion ratio (FCR); Control (CON); Thermotolerance Acclimation (TA); Probiotic Supplementation (PRO); Bunium bulbocastanum Essential Oil Supplementation (BBEO)

The superior body weight observed in the PRO group highlights the beneficial effects of probiotics on growth performance. Probiotics, such as *Lactobacillus* strains, improve gut health, nutrient absorption, and feed digestion, thereby enhancing growth efficiency. These findings align with studies by Fesseha et al. (2021) and Agustono et al. (2022)**,** where supplementation with probiotics significantly improved body weight by promoting gut microflora balance and increasing nutrient utilization. In addition, essential oil supplementation (BBEO group) significantly improved body weight compared to the control group. Essential oils possess antimicrobial and antioxidant properties, which support gut health,

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reduce oxidative stress, and enhance growth. Similar findings have been reported by Oladokun et al. (2021) and Amer et al. (2022) where essential oils, such as lavender and oregano, positively influenced growth performance by improving digestion and mitigating stress. Early-age thermal acclimation (TA group) also resulted in improved body weight compared to the control group. This suggests that controlled thermal stress during early developmental stages enhances the adaptive thermotolerance of birds, which can mitigate the negative effects of heat stress later in life. These results are consistent with findings by Nawaz et al. (2023)**,** who demonstrated that heat acclimation strategies improved growth performance and heat resilience in chickens.

Feed intake was significantly reduced in the PRO group **(**3720 ± 70 g**)** compared to the control group (3921 ± 80 g**,** $P < 0.05$). The BBEO group **(**3755 ± 74 g**)** and TA group **(**3805 ± 76 g**)** also exhibited lower feed intake compared to the control group. Reduced feed intake, particularly in the PRO group, reflects improved feed efficiency and nutrient utilization. Probiotics enhance gut health by increasing the population of beneficial bacteria (*Lactobacillus*), leading to better digestion and absorption of nutrients while lowering feed requirements for growth (Fesseha et al., 2021). The BBEO group also demonstrated improved feed intake efficiency, which can be attributed to the digestive-stimulating and antimicrobial effects of essential oils. These properties help optimize gut microbial balance, reducing energy losses associated with digestive inefficiencies, as reported by Oladokun et al. (2021)**.**

The feed conversion ratio (FCR) was significantly improved in all treatment groups compared to the control group ($P < 0.01$). The PRO group achieved the lowest FCR (1.55 ± 0.02), indicating superior feed efficiency, followed by the BBEO group **(**1.57 ± 0.03**)** and the TA group **(**1.61 ± 0.02), while the control group had the highest FCR (1.68 ± 0.02). The improved FCR in the PRO group aligns with findings by Agustono et al. (2022)**,** who reported that probiotic supplementation enhances feed efficiency by improving intestinal morphology, nutrient absorption, and gut microbial activity. Similarly, Fesseha et al. (2021) observed significant reductions in FCR when broilers were supplemented with *Lactobacillus* probiotics, which enhanced growth performance through better utilization of feed nutrients. The BBEO group's improved FCR can be attributed to the digestive and antioxidant effects of *Bunium bulbocastanum* essential oil. Essential oils improve intestinal integrity, reduce pathogenic bacterial load, and enhance nutrient absorption, ultimately improving feed efficiency. These effects are consistent with the findings of Oladokun et al. (2021) and Amer et al. (2022), were lavender and oregano essential oils improved feed conversion efficiency in broilers. Early-age thermal acclimation (TA group) also positively influenced FCR by reducing the metabolic impact of heat stress during the later stages of growth. These results corroborate the work of Nawaz et al. (2023), who demonstrated that early thermal conditioning reduces heat stress susceptibility and improves feed efficiency in broilers exposed to high temperatures.

Carcass Yield and Organ Weights

The carcass yield was significantly higher in the PRO group **(**76.2 ± 1.4%**)** compared to the control (72.3 ± 1.7%**,** $P < 0.05$). The BBEO group **(**75.6 ± 1.3%**)** and TA group **(**74.8 ± 1.5%**)** also showed significant improvements over the control (Table 2).

Table 2 : Effect on carcass yield and organ weights of acclimation and different dietary supplements

Parameter	**Control (CON)**	**TA Group**	**PRO Group**	**BBEO Group**	**P-value**
Carcass Yield (%)	72.3 ± 1.7^d	74.8 ± 1.5^c	76.2 ± 1.4^a	75.6 ± 1.3^b	< 0.05
Liver Weight (%)	2.15 ± 0.10	2.10 ± 0.09	2.05 ± 0.08	2.08 ± 0.07	0.21 (NS)
Heart Weight (%)	0.50 ± 0.02	0.51 ± 0.02	0.49 ± 0.01	0.50 ± 0.01	0.32 (NS)

Control (CON); Thermotolerance Acclimation (TA); Probiotic Supplementation (PRO); Bunium bulbocastanum Essential Oil Supplementation (BBEO)

The increased carcass yield in the PRO group can be attributed to the positive effects of probiotics on gut health, nutrient absorption, and protein deposition. Probiotics, particularly *Lactobacillus* strains, enhance feed digestion and reduce intestinal pathogens, resulting in better carcass development, as previously demonstrated by Fesseha et al. (2021) and Agustono et al. (2022)**.** The BBEO group's improved carcass yield aligns with findings from Oladokun et al. (2021) and Amer et al. (2022), where essential oils were shown to optimize nutrient utilization and reduce oxidative stress, leading to better muscle growth and meat production. Essential oils provide antimicrobial and anti-inflammatory benefits, contributing to improving overall broiler performance. The TA group also exhibited moderate improvements in carcass yield, likely due to enhanced thermotolerance achieved through early-age acclimation. This supports the findings of Nawaz et al. (2023)**,** who reported that thermal conditioning mitigates heat stress effects and enhances muscle development in broilers. Liver weights were not significantly different among the groups ($P = 0.21$), indicating that neither probiotics, essential oil supplementation, nor early thermal acclimation had adverse effects on liver development. The liver, as a central organ for metabolism, remained physiologically stable across all treatments, reflecting the safety of the applied interventions. These results are consistent with studies by Oladokun et al. (2021) and Agustono et al. (2022), who reported no adverse effects of probiotics or essential oils on organ weights in broilers.

Heart weights were also not significantly different among the groups ($P = 0.32$). This suggests that none of the treatments imposed additional cardiovascular stress or adverse effects on heart development. Probiotics and essential oils are known

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to promote physiological stability, and early thermal acclimation did not negatively influence cardiac health. Similar findings have been reported by Amer et al. (2022), highlighting the safety of essential oil supplementation in broilers.

Meat quality

pH, WHC, and cooking loss

Table 3 shows the initial pH (45 minutes' post-mortem) showed no significant differences ($P = 0.12$) among the groups, indicating similar rates of post-slaughter glycolysis. However, significant differences were observed in the ultimate pH (24 hours' post-mortem, $P < 0.05$). The BBEO group exhibited the highest ultimate pH (5.95 ± 0.05), followed by the PRO group (5.90 ± 0.06) and the TA group (5.88 ± 0.04), while the control group had the lowest value (5.79 ± 0.07).

Table 3: Effect of Treatments on Meat pH, WHC, and Cooking Loss in Broilers

Parameter	Control (CON)	TA Group	PRO Group	BBEO Group	P-value
Initial pH (45 min)	6.12 ± 0.05^{a}	6.10 ± 0.06^{a}	6.08 ± 0.04^{a}	6.07 ± 0.03^{a}	0.12 (NS)
Ultimate pH (24 h)	5.79 ± 0.07^{b}	5.88 ± 0.04^{ab}	5.90 ± 0.06^{a}	5.95 ± 0.05^{a}	< 0.05
WHC (%)	71.2 ± 1.8^{d}	74.5 ± 1.3^{c}	76.8 ± 1.5^{b}	78.4 ± 1.2^{a}	< 0.01
Cooking Loss (%)	19.8 ± 0.8^{a}	17.5 ± 0.7^{b}	16.1 ± 0.6^{c}	15.3 ± 0.5^{c}	< 0.01

Control (CON); Thermotolerance Acclimation (TA); Probiotic Supplementation (PRO); Bunium bulbocastanum Essential Oil Supplementation (BBEO); Water Holding Capacity (WHC)

Higher ultimate pH values indicate reduced muscle glycogen depletion, likely due to lower pre-slaughter stress and improved antioxidant status in the BBEO and PRO groups. This aligns with the findings of Beauclercq et al. (2022), who highlighted the relationship between ultimate pH and muscle metabolism, showing that improved pH levels are associated with reduced stress and better lipid profiles. Similarly, Liu et al. (2022) reported that ultimate pH serves as a reliable indicator of meat quality and freshness, suggesting that the BBEO and PRO groups maintained better post-mortem muscle integrity. Water holding capacity (WHC) was significantly improved in all treatment groups compared to the control ($P < 0.01$), with the BBEO group achieving the highest WHC (78.4 ± 1.2%), followed by the PRO group (76.8 ± 1.5%) and the TA group (74.5 ± 1.3%). The control group exhibited the lowest WHC (71.2 ± 1.8%). Improved WHC is associated with enhanced protein functionality and reduced protein denaturation, which limits water loss in meat. This is supported by Warner (2023), who emphasized the importance of WHC in determining juiciness and overall eating quality of meat. Essential oils, such as *Bunium bulbocastanum*, contain strong antioxidants that mitigate oxidative stress and prevent muscle protein degradation, leading to improved water retention. Similarly, probiotics enhance nutrient utilization and protein deposition, as observed in the PRO group. These findings align with Cornet et al. (2021), who demonstrated that improved WHC is influenced by structural protein interactions and oxidative stability. Cooking loss was significantly reduced in all treatment groups compared to the control ($P < 0.01$). The BBEO group had the lowest cooking loss (15.3 ± 0.5%), followed by the PRO group (16.1 ± 0.6%) and the TA group (17.5 ± 0.7%), while the control group exhibited the highest cooking loss (19.8 ± 0.8%). Reduced cooking loss reflects improved water retention during the cooking process, resulting in juicier and more tender meat. According to Warner (2023), cooking loss is closely linked to WHC and muscle protein integrity. The significant reductions in cooking loss observed in the BBEO and PRO groups are likely due to the antioxidant effects of essential oils and probiotics, which stabilize muscle proteins and prevent lipid oxidation. These results align with Djilali et al. (2016), who reported that lipid oxidation leads to structural damage and increased water loss, negatively affecting meat quality. The improved cooking loss in the BBEO group confirms the strong antioxidant capacity of *Bunium bulbocastanum* essential oil, which protects meat quality by reducing oxidative stress.

Meat composition

Demonstrated in Table 4, crude protein content was significantly higher in the treatment groups compared to the control ($P < 0.01$). The BBEO group exhibited the highest crude protein content (23.1 ± 0.4%), followed by the PRO group (22.8 ± 0.5%) and the TA group (22.1 ± 0.5%), while the control group had the lowest protein content (21.5 ± 0.6%).

Table 4: Effect of broiler chicks' acclimation and dietary supplementation on crude protein, fat %, MDA and PUFA composition

Parameter	Control (CON)	TA Group	PRO Group	BBEO Group	P-value
Crude Protein (%)	21.5 ± 0.6^{d}	22.1 ± 0.5^{c}	22.8 ± 0.5^{b}	23.1 ± 0.4^{a}	< 0.01
Fat (%)	3.1 ± 0.4^{a}	2.9 ± 0.3^{ab}	2.6 ± 0.3^{b}	2.4 ± 0.3^{b}	< 0.05
MDA Levels (mg/kg)	0.85 ± 0.05^{a}	0.60 ± 0.04^{b}	0.53 ± 0.03^{bc}	0.45 ± 0.02^{c}	< 0.01
PUFA Content (%)	15.4 ± 0.8^{d}	18.1 ± 0.9^{c}	21.5 ± 1.0^{b}	24.3 ± 1.1^{a}	< 0.01

Control (CON); Thermotolerance Acclimation (TA); Probiotic Supplementation (PRO); Bunium bulbocastanum Essential Oil Supplementation (BBEO); Malondialdehyde (MDA); Polyunsaturated fatty acids (PUFA)

The increase in crude protein content in the BBEO group can be attributed to the antioxidant and antimicrobial properties of *Bunium bulbocastanum* essential oil, which enhance the nutrient absorption and protein deposition by maintaining gut integrity and reducing oxidative damage (Amer et al., 2022**;** Oladokun et al., 2021). Similarly, probiotics in the PRO group improve protein synthesis by enhancing gut health, promoting beneficial microflora, and optimizing nutrient utilization (Fesseha et al., 2021**;** Agustono et al., 2022). Early-age thermal acclimation (TA group) also resulted in higher crude protein levels compared to the control, likely due to its role in mitigating heat stress effects, thereby reducing protein catabolism and improving nutrient retention (Nawaz et al., 2023). The fat content was significantly lower in the BBEO and PRO groups compared to the control group ($P < 0.05$). The BBEO group had the lowest fat content (2.4 ± 0.3%), followed by the PRO group (2.6 ± 0.3%) and the TA group (2.9 ± 0.3%), while the control group recorded the highest fat content (3.1 ± 0.4%).The reduction in fat content in the BBEO group can be attributed to the lipolytic and antioxidant effects of essential oils, which enhance lipid metabolism and prevent excessive fat deposition (Amer et al., 2022; Djilali et al., 2016). Probiotics similarly improve energy utilization and lipid metabolism by modulating gut microbiota and reducing fat accumulation (Fesseha et al., 2021). Malondialdehyde (MDA) levels, an indicator of lipid oxidation, were significantly reduced in the treatment groups compared to the control ($P < 0.01$). The BBEO group exhibited the lowest MDA levels (0.45 ± 0.02 mg/kg), followed by the PRO group (0.53 ± 0.03 mg/kg) and the TA group (0.60 ± 0.04 mg/kg). The control group showed the highest MDA levels (0.85 ± 0.05 mg/kg). The lower MDA levels in the BBEO group highlight the strong antioxidant capacity of *Bunium bulbocastanum* essential oil, which protects lipids from oxidative damage, thereby enhancing meat quality and shelf life (Amer et al., 2022; Warner, 2023). Probiotics in the PRO group also reduced lipid oxidation, likely by improving gut health and overall antioxidant status (Fesseha et al., 2021). Early thermal acclimation (TA group) contributed to moderate reductions in MDA levels, as thermal adaptation reduces oxidative stress during heat exposure (Nawaz et al., 2023).

The PUFA content was significantly higher in all treatment groups compared to the control ($P < 0.01$). The BBEO group had the highest PUFA content (24.3 ± 1.1%), followed by the PRO group (21.5 ± 1.0%) and the TA group (18.1 ± 0.9%), while the control group had the lowest PUFA content (15.4 ± 0.8%).

Higher PUFA levels in the BBEO group reflect the role of essential oils in reducing lipid peroxidation and preserving unsaturated fatty acids, thereby enhancing the nutritional value of meat (Djilali et al., 2016**;** Amer et al., 2022). Probiotics similarly improved the fatty acid profile by promoting beneficial gut microbiota that influence lipid metabolism and PUFA synthesis (Fesseha et al., 2021). Early thermal acclimation also led to an increase in PUFA content, likely due to its role in reducing heat-induced oxidative stress and lipid degradation (Nawaz et al., 2023).

Conclusion

This study demonstrates that early-age thermotolerance acclimation, probiotics, and *Bunium bulbocastanum* essential oil supplementation effectively mitigate the adverse effects of heat stress on broilers while improving growth performance, carcass traits, and meat quality. The probiotic group (PRO**)** achieved the highest growth performance, with significant improvements in the final body weight and feed conversion ratio, highlighting the role of probiotics in enhancing gut health, nutrient absorption, and feed efficiency. The essential oil group (BBEO) exhibited superior meat quality, including higher crude protein content, reduced fat deposition, improved water holding capacity (WHC), lower cooking loss, and enhanced oxidative stability, as indicated by reduced MDA levels and increased PUFA content. These benefits can be attributed to the strong antioxidant and antimicrobial properties of *Bunium bulbocastanum* essential oil. The thermotolerance acclimation group (TA) provided moderate improvements in growth and meat quality, demonstrating the effectiveness of early-age thermal conditioning in enhancing heat resilience. The integration of *Bunium bulbocastanum* essential oil and probiotics into broiler diets, along with early-age thermal acclimation, presents a promising approach to improving broiler resilience, productivity, and meat quality under heat stress conditions. These findings offer practical solutions for sustainable poultry production in heat-challenged environments, ensuring both producer profitability and consumer satisfaction.

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