Effects of Probiotics and Aromatic Plant Blends on Productive Performance and Meat Quality of Broiler Chicks under Heat Stress Conditions

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Abstract

Heat stress (HS) represents a major challenge in modern poultry production. It causes physiological imbalances that adversely affect the growing performance and meat quality of broilers. This study evaluated the effectiveness of supplementing broiler diets with two types of probiotics (Bacillus subtilis and a Lactobacillus blend) and two aromatic plant blends (Thymus vulgaris + Rosmarinus officinalis and Syzygium aromaticum + Cinnamomum zeylanicum) under heat stress conditions. A total of 300 one-day-old broiler chicks with uniform body weight were randomly assigned to six dietary treatment groups in a completely randomized design (CRD). Each treatment included five replicate floor pens with 10 birds per pen. The experimental groups were negative control (HS), Probiotic A (B. subtilis), Probiotic B (Lactobacillus), Herbal A (T. vulgaris + R. officinalis), Herbal B (S. aromaticum + C. verum), and a synbiotic group combining the most promising probiotic and herbal mixture. The experiment lasted for 35 days, with HS (32-34 °C, 8 hours a day) induced between days 21 and 35. The findings demonstrated that final body weight increased by approximately 14%, and the feed conversion ratio (FCR) improved from 1.89 to 1.63 in the synbiotic group compared to the control ($P < 0.05$), with no marked differences in feed intake among treatments. Furthermore, carcass characteristics were enhanced, particularly breast yield. Meat quality also improved, as evidenced by reduced TBARS values after 10 days of refrigerated storage, with the aromatic plant blends exhibiting the greatest antioxidant activity. The fatty acid profile was positively altered, showing an increased presence of polyunsaturated fatty acids, which indicates a decrease in lipid peroxidation. Supplementing broiler diets with probiotics and aromatic plant blends, particularly when used in combination, effectively prevents the adverse effects of heat stress. This dietary strategy enhances the productive performance of broilers and secures the stability of the oxidative properties and overall meat quality.

Keywords: Broiler chicks, Heat stress, Probiotics, Aromatic plants, Productive performance, Meat quality.

Introduction

Poultry production is an important contributor to global food security because it provides a relatively efficient source of high-quality animal protein [21]. However, the increasing frequency of heatwaves associated with climate change threatens the sustainability and profitability of broiler production. Broilers are particularly vulnerable to heat stress (HS) because rapid growth and high metabolic heat production limit their capacity to dissipate heat. Consequently, chronic HS reduces

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feed intake and growth performance, worsens feed conversion, disrupts intestinal function, and increases oxidative damage [40,2]. It also compromises meat quality by accelerating lipid oxidation, particularly in polyunsaturated fatty acids, thereby reducing oxidative stability, shelf life, and consumer acceptability [29,32].

Nutritional strategies are therefore needed because cooling and other environmental interventions may be costly or impractical under commercial conditions, while the use of antibiotic growth promoters has been restricted because of antimicrobial-resistance and food-safety concerns [34,36]. Probiotics are promising alternatives because they can support intestinal microbial balance and barrier function, inhibit enteric pathogens, improve nutrient utilization, and enhance broiler performance during stress [27,17]. The efficacy of a probiotic is strain-dependent. Spore-forming Bacillus subtilis is stable during feed processing and passage through the upper gastrointestinal tract, whereas Lactobacillus strains can contribute to an acidic intestinal environment that limits the growth of acid-sensitive pathogens [1,8]. These distinct properties justify comparing these probiotic types under chronic HS rather than treating probiotics as a functionally uniform group.

Phytogenic feed additives provide a complementary nutritional approach. Aromatic plants such as Thymus vulgaris and Rosmarinus officinalis are rich in phenolic compounds with antioxidant activity, whereas Syzygium aromaticum and Cinnamomum zeylanicum provide phytochemicals with pronounced antimicrobial activity and potential to support digestive processes [16,32]. In broilers, phytogenic supplementation has been associated with improved productive performance and greater oxidative stability of meat [25]. Thus, the relevance of these plants to heat-stressed broilers lies mainly in three practical outcomes: protection against oxidative damage, moderation of undesirable intestinal microorganisms, and support of digestion and production.

Combining probiotics with phytogenic additives is scientifically justified by their potentially complementary modes of action. Probiotics primarily act through the intestinal microbiota and epithelial barrier, whereas phytogenic compounds may provide direct antioxidant activity together with complementary antimicrobial and digestion-supporting effects. Previous studies have consequently proposed that combining these additive classes may provide broader protection against complex stressors such as HS than either class alone [29,17]. Nevertheless, a synergistic response should not be assumed because it may depend on the probiotic strain, the botanical composition, dosage, and the physiological response being measured. Direct evidence comparing specific probiotic strains with functionally distinct phytogenic blends under chronic HS remains limited.

The principal research gap is the lack of a comprehensive comparison, under chronic HS, of a spore-forming probiotic, a lactic acid bacterial blend, and phytogenic combinations selected for different functional profiles, together with an evaluation of their combined effects on both production and meat quality. In particular, the interaction between the most effective probiotic and the most appropriate herbal blend has not been sufficiently defined across growth performance, carcass characteristics, meat composition, and oxidative stability [30].

Accordingly, this study evaluated the effects of Bacillus subtilis, a Lactobacillus blend, two phytogenic blends—Herbal A (T. vulgaris + R. officinalis) and Herbal B (S. aromaticum + C. zeylanicum)—and

selected probiotic–phytogenic combinations in broilers exposed to chronic HS. The responses measured were body weight, feed intake, feed conversion ratio, carcass traits, meat physicochemical characteristics, breast-meat fatty acid composition, and oxidative stability [15].

Material and Methods

Experimental location, plant materials, and probiotic preparations

Day-old broiler chicks were obtained from a commercial hatchery (Modern Al-Salam Poultry Hatchery, Al-Hillah City, Babil Governorate, Iraq). The chicks were clinically healthy and had uniform body weight at the start of the experiment. The medicinal plants used in this study were obtained from the College of Medicinal and Industrial Plants, Kirkuk University, Kirkuk, Iraq. The plant materials—comprising dried leaves of Thymus vulgaris L. and Rosmarinus officinalis L. (Herbal A), as well as dried flower buds of Syzygium aromaticum L. and dried inner bark of Cinnamomum zeylanicum Blume (Herbal B)—were processed as standardized hydroalcoholic extracts following the methodology described [16,41]. Briefly, the shade-dried plant parts were pulverized to pass through a 1-mm mesh screen and extracted with 70% aqueous ethanol (1:10 w/v ratio) for 48 h at 40 °C under constant agitation. The extract mixture was filtered through Whatman No. 1 paper, evaporated under reduced pressure using a rotary evaporator, and freeze-dried to obtain a dry extract powder, which was stored at −20 °C until inclusion into the experimental diets. The commercial probiotic product METABO® (PVS Group, India), used as Probiotic A (Pro A), was incorporated into the experimental diets at the specified inclusion level. Probiotic B (Pro B), consisting of a Lactobacillus blend containing Lactobacillus acidophilus and Lactobacillus plantarum, was obtained from the Department of Microbiology, College of Agriculture, University of Karbala, Karbala, Iraq, and prepared according to standard microbiological procedures before use. The in vivo experiment was conducted at the Poultry Research Facility, Department of Animal Production, College of Agriculture, University of Karbala, Iraq. The facility has a closed-house system with automated environmental control, including pad cooling systems, exhaust fans, and programmable heating units, which enabled accurate control of ambient temperature and relative humidity. All experimental procedures, animal handling, and care were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Karbala (Approval No. UOK.Agri.11.2026), ensuring compliance with internationally accepted guidelines for animal welfare in agricultural research.

Experimental Design, Birds, and Diets

Three hundred one-day-old, unsexed Ross 308 broiler chicks were purchased from a commercial broiler hatchery. Upon arrival, each of the chicks was weighed separately to ensure the initial uniformity of the body size and then randomly assigned to six dietary treatment groups in a completely randomized design (CRD). Each treatment had 5 replicate floor pens, with 10 birds in each pen. The pens were supplied with new shavings of wood as bedding. Each pen had a tube feeder and a bell drinker so that they could have ad libitum access to feed and water during the entire duration of the experiment (35 days). The six experimental treatments were defined as follows:

(1) Negative Control (Control): Birds were fed the basal diet and subjected to heat stress (HS) without any feed additives.

(2) Probiotic A (Pro A): Birds fed the basal diet supplemented with *Bacillus*

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subtilis (commercial product, providing 1 × 10^9 CFU/kg of feed) + HS.

(3) Probiotic B (Pro B): Birds were fed the basal diet supplemented with a *Lactobacillus blend* (containing *L. acidophilus and L. plantarum,* providing 1 × 10^9 CFU/kg of feed) + HS.

(4) Herbal A (Herb A): Birds fed the basal diet supplemented with a blend of *Thymus vulgaris* (thyme) and *Rosmarinus officinalis* (rosemary) at a 1:1 ratio, added at 5 g/kg of feed + HS.

(5) Herbal B (Herb B): Birds fed the basal diet supplemented with a blend of *Syzygium aromaticum* (Clove) and *Cinnamomum zeylanicum* (Cinnamon) at a 1:1 ratio, added at 5 g/kg of feed + HS.

(6) Synbiotic (Syn): Birds were fed the basal diet supplemented with the combination of the best-performing probiotic (Pro A) and the herbal blend thyme and rosemary (Herb A) at the same inclusion levels used in individual treatments under heat stress conditions.

The basal diets were carefully designed to address or slightly exceed the nutritional needs prescribed to Ross 308 broilers in three different growth stages: starter (days 1-10), grower (days 11-24), and finisher (days 25-35). The basic ingredients were yellow corn, soybean meal, poultry fat, and molasses with additional vitamins and minerals. Table 1 includes the detailed ingredients and calculated and analyzed nutrient composition of the basal diets of all three phases in a comprehensive manner [42]**.**

Table 1. Ingredients and nutrient composition (calculated and analyzed) of the basal diets fed to broiler chicks s during the starter, grower, and finisher phases

Item	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-35 d)
Ingredients (%)			
Yellow Corn	55.5	58.0	60.5
Soybean Meal (48% CP)	32.0	28.5	24.0
Barley	0.0	5.0	8.0
Poultry Fat	5.5	4.5	3.5
Molasses	2.0	1.5	1.5
Limestone (CaCO3)	1.2	1.0	1.0
Dicalcium Phosphate	1.5	1.0	0.8
Vitamin Premix[1]	1.0	0.8	0.5
Mineral Premix[2]	1.0	0.8	0.5
Salt (NaCl)	0.3	0.3	0.2
Total	100.0	100.0	100.0
Metabolizable Energy (ME, kcal/kg)	3050.0 / 3045.0	3150.0 / 3148.0	3200.0 / 3198.0
Crude Protein (CP, %)	23.5 / 23.2	21.0 / 20.8	19.5 / 19.2
Ether Extract (EE, %)	6.8 / 6.5	6.2 / 6.0	5.8 / 5.6
Crude Fiber (CF, %)	3.2 / 3.1	3.5 / 3.4	3.8 / 3.7
Calcium (Ca, %)	1.0 / 1.02	0.95 / 0.93	0.9 / 0.88
Available Phosphorus (Av. P, %)	0.48 / 0.46	0.42 / 0.4	0.38 / 0.36
Methionine (Met, %)	0.52 / 0.5	0.48 / 0.46	0.45 / 0.43
Lysine (Lys, %)	1.28 / 1.25	1.15 / 1.12	1.05 / 1.02
Methionine + Cystine (Met+Cys, %)	0.95 / 0.92	0.88 / 0.85	0.82 / 0.8

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Arginine (Arg, %)	1.45 / 1.42	1.32 / 1.3	1.25 / 1.22
Threonine (Thr, %)	0.78 / 0.76	0.72 / 0.7	0.68 / 0.66
Tryptophan (Trp, %)	0.22 / 0.21	0.2 / 0.19	0.18 / 0.17

Heat Stress Challenge Protocol

To recreate the conditions of chronic heat stress that are characteristic of summer months in dry areas, a special thermal challenge protocol was introduced. Daily 1 to 20, birds were kept under normal thermoneutral conditions as per the Ross 308 management guidelines (initially kept at 32°C on day 1 with a gradual lowering of the temperature of 0.3 °C/day by day until they reached 24°C on day 20). The chronic heat stress protocol was administered starting on day 21 and continuing until the end of the experiment on day 35. The ambient temperature of the facility was set artificially to 32-34 °C over a period of 8 hours per day (09: 00 AM to 05: 00 PM). Relative humidity was maintained at 55-60 % during this heat challenge period. The temperature was set back to the thermoneutral temperature of 24°C, in the remaining 16 hours of the day (05:00 pm 09:00 am). This was a cyclic heating model, which was created to induce physiological stress, but not excess acute mortality. Heat stress protocol was applied according to Fouad and El-Senousey [11].

Measurement of Productive Performance

The trial was closely monitored to track productive performance parameters. Live body weight (BW) of the birds in each pen was recorded once a week. The feed intake (FI) was calculated as the total weight of feed fed and the weight of feed left in feeders at the end of each week. Based on these measurements, the feed conversion ratio (FCR) was calculated as the ratio of total feed consumed to the total weight gain per pen [6].

Carcass Traits Evaluation

By the end of the feeding trial on day 35, two birds per replication pen (10 birds per treatment), which were the average body weights of the pen, were chosen to evaluate their carcasses. Selected birds were fasted for 8–12 h (with free access to water) before slaughter to empty the gastrointestinal tract of contents, weighed separately to determine the pre-slaughter weight, and the jugular vein and carotid artery were severed following conventional commercial slaughter techniques. The weight of the hot carcass was measured after bleeding, scalding, plucking, and total evisceration. Dressing percentage was computed as the hot carcass weight/pre-slaughter live weight. The large commercial incisions, the breast muscle (pectoralis major and minor), and the thigh muscle were dissected and weighed. The yields of the breast and thigh were expressed as percentages of the hot carcass weight. All slaughter procedures were conducted in accordance with institutional animal care and welfare guidelines approved by the IACUC.

Physicochemical Analysis and Meat Quality.

After the carcass assessment, right breast muscle (Pectoralis major) samples were taken out of all slaughtered birds to undertake a complete analysis of meat quality. Ultimate pH: A portable digital pH meter with a piercing glass electrode inserted directly into the core of muscle tissue was used to measure the pH of the breast meat 24 hours after death (pH24h). Meat Color: Instrumental color of the surface of the breast meat was measured 24 hours after death using a Minolta Chroma Meter (CR-400). The color coordinates were represented in the CIELAB system with the Lightness (L),

Redness (a), and Yellowness (b). Chemical Composition: The proximate chemical composition of the breast meat with water, crude protein, and extract (intramuscular fat) was established under the standard procedures of the Association of Official Analytical Chemists [35].

The determination of fatty acids.

The intramuscular fat that was extracted from the breast muscle was analyzed through Gas Chromatography (GC). The Folch method (chloroform: methanol, 2:1 v/v) was used to extract total lipids. The lipids extracted were further trans esterified to fatty acid methyl esters (FAMEs). The FAMEs were introduced into a Gas Chromatograph that had a flame ionization detector (FID) and a capillary column. The identification of the individual fatty acids was achieved through comparing their retention times with those of the actual commercial standards. These findings were given as a share of the total identified fatty acids, and they were saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) [10].

The oxidative stability was evaluated by the oxidative stability (TBARS Assay).

The oxidative stability of the breast meat in refrigerated storage conditions was determined by determining the Thio barbituric Acid Reactive Substances (TBARS) level, which measures the concentration of malondialdehyde (MDA), one of the secondary products of lipid peroxidation. Fresh meat sampler was minced, placed in oxygen-permeable polyethylene bags, and kept in a refrigerator at 4 o C. TBARS test was conducted at days 0, 5, and 10 of storage. Absorbance of the complex that was formed was spectrophotometrically measured, and TBARS were reported in terms of milligrams of MDA per kilogram of meat [9].

Statistical Analysis

All obtained data were thoroughly analyzed statistically to ascertain the significance of treatment effects. The productive performance, carcass traits, physicochemical properties, and fatty acid profiles data were processed by the one-way Analysis of Variance (ANOVA) procedure that is suitable for a Completely Randomized Design (CRD), and the General Linear Model (GLM) procedure of the SAS software (Version 9.4, SAS Institute Inc., Cary, NC, USA). Where the main effects were found to be significant ($P < 0.05$), the differences between the treatment means were partitioned and compared using Tukey's HSD. In the case of the oxidative stability (TBARS) data, where multiple measurements of the same data were done at different intervals on the same units of the experiment, a Repeated Measures ANOVA was used to assess the primary effects of dietary treatment, storage time, and their interaction.

Results

Growth Performance

The productive performance parameters of broiler chicks s subjected to chronic heat stress and fed various dietary supplements are systematically detailed in Table 2. The use of thermal stress largely affected the growth pattern of the negative control group, which was supplemented and had the lowest final body weight and the least efficient ratio of feed to weight. On the other hand, dietary interventions (both probiotic strains, as well as aromatic plant mixtures) had a strongly significant ($P < 0.05$) ameliorative effect on the following growth parameters. There were significant improvements in final body weight in all supplemented groups compared to the control. The Synbiotic group, which received the combination of *Bacillus subtilis* and the (thyme (Thymus vulgaris) /rosemary extract, had a peak final body weight of 2450.3 g with statistically significant superiority over both the

control and the individual herbal treatments. The dietary treatments had a tremendous positive effect in terms of feed utilization efficiency. As demonstrated in the Fig 1., Feed Conversion Ratio (FCR) was greatly streamlined in all the treated groups compared to the control of 1.89. Synbiotic regimen has shown the greatest FCR of 1.63, highlighting a significant improvement in nutrient absorption and use in unfavorable thermal conditions. Moreover, *Bacillus subtilis* supplementation (Pro A) showed very high utilization of feed (1.69), and it was statistically significant over *Lactobacillus* blend (Pro B).

Table 2. Effect of dietary supplementation with probiotics and aromatic plant blends on productive performance of broiler chicks s under heat stress

Treatment	Initial Weight (g)	Final Weight (g)	Feed Intake (g)	FCR
Negative Control (HS)	42.1 ± 0.5	2150.5 ± 45^{c}	3980.5 ± 60^{a}	1.89 ± 0.04^{a}
Probiotic A (B. subtilis)	42.5 ± 0.6	2380.2 ± 50^{ab}	3950.2 ± 55^{a}	1.69 ± 0.03^{c}
Probiotic B (Lactobacillus)	42.3 ± 0.5	2345.8 ± 48^{b}	3965.4 ± 58^{a}	1.72 ± 0.03^{bc}
Herbal A (thyme +Rosemary)	42.2 ± 0.4	2310.4 ± 46^{b}	3910.8 ± 32^{b}	1.72 ± 0.02^{bc}
Herbal B (Clove+Cinnamon)	42.4 ± 0.5	2295.6 ± 44^{b}	3925.5 ± 50^{b}	1.74 ± 0.03^{b}
Synbiotic (Best Pro+Herb)	42.6 ± 0.6	2450.3 ± 52^{a}	3940.1 ± 54^{a}	1.63 ± 0.02^{c}

Data are presented as means ± SD (n=5 replicates per treatment). Mean within some column bearing different superscript letters differ significantly (P<0.05). FCR = Feed Conversion Ratio.

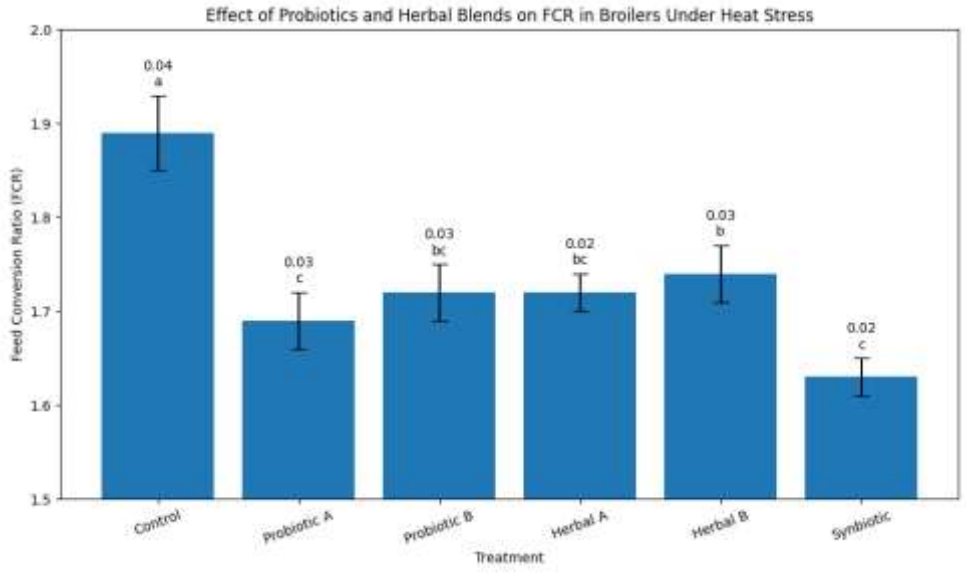


Fig 1. Feeding broiler chicks s under heat stress with probiotics and aromatic plant blends. Effect of dietary supplementation with probiotics and aromatic plant blends on Feed Conversion Ratio (FCR) in broiler chicks s under heat stress. The Synbiotic (Pro A + Herb A)

showed the highest FCR (1.63), which was 13.8% higher than the control group, which was heat-stressed (1.89). This increased feed efficiency indicates greater nutrient digestibility and absorption brought on by the synergistic effect of *Bacillus subtilis* (enhanced gut microbiota and intestinal morphology) and thyme /rosemary extract (stimulated endogenous enzyme secretion).

Carcass Traits

Table 3. explains in detail the effects of dietary manipulations on the key carcass characteristics and yield parameters. The experimental additives have demonstrated a significant ($P < 0.05$) percentage of dressing, which is a significant economic parameter in poultry processing. All the cohorts with supplements showed high dressing yields compared with the stress on the thermally negative control group, which had a recorded baseline of 68.5% dressing. The highest percentage of dressing was 73.5%, which was achieved by the synergistic approach used in the Synbiotic group. In addition, the dietary interventions gave strong defense to skeletal muscle growth against the already established catabolic effects of heat stress, which is evident in the proportionate weights of the high-value commercial cuts. The relative yield of the breast muscle was greatly increased in all the treated groups. The Synbiotic group recorded a remarkable breast yield of 28.5, which is much higher physiologically compared to the 24.5% yield of the control group. The thigh yield rated parallel positive response, which supports the effectiveness of these nutritional interventions in maintaining and accelerating muscle mass accretion in times of exhaustive thermal load.

Table 3. Carcass traits of broiler chicks s as influenced by dietary supplementation with probiotics and aromatic plant blends under heat stress

Treatment	Dressing Yield (%)	Breast Yield (%)	Thigh Yield (%)
Negative Control (HS)	68.5 ± 1.2^{c}	24.5 ± 0.8^{c}	20.1 ± 0.7^{c}
Probiotic A (*B. subtilis)*	72.1 ± 1.0^{ab}	27.8 ± 0.9^{ab}	22.5 ± 0.8^{ab}
Probiotic B (*Lactobacillus*)	71.5 ± 1.1^{b}	27.2 ± 0.8^{b}	22.1 ± 0.7^{b}
Herbal A(thyme+Rosemary)	71.8 ± 1.0^{b}	27.5 ± 0.9^{ab}	22.3 ± 0.8^{ab}
Herbal B (Clove+Cinnamon)	71.2 ± 1.1^{b}	26.9 ± 0.8^{b}	21.8 ± 0.7^{b}
Synbiotic (Best Pro+Herb)	73.5 ± 1.2^{a}	28.5 ± 1.0^{a}	23.1 ± 0.9^{a}

The values are given in the form of mean and standard deviation. Means in the same column that have different superscript letters (a–c) differ significantly at $P < 0.05$. HS: heat stress; Probiotic A: *Bacillus subtilis*; Probiotic B: *Lactobacillus spp*.; Herbal A: thyme (*Thymus vulgaris*) + rosemary (*Rosmarinus officinalis*); Herbal B: clove (*Syzygium aromaticum*) + cinnamon (*Cinnamomum zeylanicum*); Synbiotic: combination of probiotic and herbal treatments.

Physicochemical Properties of Meat

Table 4 shows systematically the basic physicochemical properties and the proximate biochemical composition of the harvested breast meat. The pH (pH 24h) analysis revealed that the experimental samples had slight differences, but all the values were within the acceptable normal physiological pH of poultry meat (5.75 - 6.02). This key observation means that neither dietary interventions nor heat stress protocol triggered any of the most extreme myopathic aberrations, including Pale, Soft, and Exudative (PSE) meat. The instrumental measure of the meat color coordinates showed that the dietary

treatments had a considerable effect on the visual appearance of the tissue. As a rule, the supplemented groups also had lower lightness (L) values, but higher redness (a) values as compared to the normal light meat of the heat-stressed control group. This change implies a physical enhancement of the aesthetic and tactile aspects of the product [2]. Additionally, the stringent examination of the immediate chemical structure revealed that there were major ($P < 0.05$) metabolic changes that were instigated by dietary supplements. The concentration of crude protein in the breast musculature was greatly increased in each of the treated groups, with a high of 24.1% in the Synbiotic group, with a significant change as compared to the 21.5 % in the control. In an inverse correlation, intramuscular fat concentration (ether extract) was reduced significantly in the supplemented groups. The Synbiotic one had the lowest rate of fat deposition (2.2) than the control (3.5). This change in composition is a good indication of a very positive metabolic redistribution of nutrients, with a focus on functional protein accretion, instead of lipid excess buildup during conditions of environmental stress.

Table 4. Physicochemical properties and proximate composition of breast meat from broiler chicks s supplemented with probiotics and aromatic plant blends under heat stress

Treatment	pH (24h)	Color L	Color a*	Color b*	Protein (%)	Fat (%)
Negative Control (HS)	.75 ± 0.05^{c}	4.2 ± 1.3^{a}	1 ± 0.2^{c}	.5 ± 0.6^{a}	1.5 ± 0.7^{c}	3.5 ± 0.2^{a}
Probiotic A *(B. subtilis*)	.95 ± 0.06^{ab}	0.1 ± 1.1^{b}	5 ± 0.3^{b}	.2 ± 0.5^{b}	3.2 ± 0.8^{b}	2.8 ± 0.2^{b}
Probiotic B (*Lactobacillus*)	.92 ± 0.05^{b}	0.5 ± 1.2^{b}	3 ± 0.3^{b}	.5 ± 0.5^{b}	2.9 ± 0.7^{b}	2.9 ± 0.2^{b}
HerbalA (thyme +Rosemary)	.98 ± 0.06^{ab}	9.8 ± 1.1^{bc}	.8 ± 0.3^{ab}	3 ± 0.4^{bc}	3.5 ± 0.8^{b}	2.5 ± 0.2^{bc}
Herbal B (Clove+Cinnamon)	.94 ± 0.05^{b}	50.2 ± 1.1^{b}	4 ± 0.2^{b}	.1 ± 0.5^{b}	3.1 ± 0.7^{b}	2.7 ± 0.2^{b}
Synbiotic (Best Pro+Herb)	.02 ± 0.07^{a}	9.1 ± 1.0^{c}	.1 ± 0.3^{a}	5 ± 0.4^{c}	24.1 ± 0.9^{a}	2.2 ± 0.1^{c}

Data are presented as means ± SD (n = 5 replicates per treatment). Means within the same column bearing different superscript letters are significantly different ($P < 0.05$). L*, a*, and b* represent CIELAB color coordinates (Lightness, Redness, and Yellowness, respectively). pH 24h = ultimate pH measured 24 hours post-mortem. The supplemented groups maintained normal pH values, preventing PSE-like meat defects.

Fatty Acid Profile

Detailed chromatographic study of the fatty acid composition of the intramuscular fat of the breast muscle is outlined in Table 5. The planned intake of probiotics and operational aromatic plant mixtures using diet had a profound control over the tissue lipid profile, and thus substantially improved its nutritional and functional properties. The proportionality of saturated fatty acids (SFA) was considerably ($P < 0.05$) reduced in all the supplemented groups in comparison to the high levels in the control (35.5%). It is interesting to note that the Synbiotic group had the greatest reduction, as it had the lowest concentration of SFA (28.5%). At the same time, the proportion of desirable polyunsaturated fatty acids (PUFA) that are relatively high was also considerably increased within the treated groups. The use of plant blends of aromatic plants

(Herbal A and Herbal B), and the Synbiotic treatment was found to be particularly effective in increasing the levels of PUFA. A direct result of these compositional changes was that the PUFA/SFA ratio, which is an important nutritional index used to determine the healthfulness of dietary lipid sources consumed, was greatly optimized. The Synbiotic cohort (0.93) and the Herbal A cohort (0.89) had the most physiologically desirable ratios, showing a significant and statistically significant increase over the metabolically impaired heat-stressed control group (0.63).

Table 5. Fatty acid profile of breast muscle from broiler chicks s supplemented with probiotics and aromatic plant blends under heat stress

Treatment	SFA (%)	MUFA (%)	PUFA (%)	PUFA/SFA
Negative Control (HS)	35.5 ± 1.1^{a}	42.1 ± 0.9^{c}	22.4 ± 0.8^{c}	0.63 ± 0.03^{c}
Probiotic A (*B. subtilis)*	31.2 ± 1.0^{b}	43.5 ± 0.8^{b}	25.3 ± 0.7^{b}	0.81 ± 0.02^{b}
Probiotic B (*Lactobacillus*)	31.8 ± 1.0^{b}	43.1 ± 0.8^{b}	25.1 ± 0.7^{b}	0.79 ± 0.02^{b}
Herbal A (thyme+Rosemary)	29.5 ± 0.9^{c}	44.2 ± 0.8^{ab}	26.3 ± 0.8^{ab}	0.89 ± 0.03^{a}
Herbal B (Clove+Cinnamon)	30.2 ± 1.0^{bc}	43.8 ± 0.8^{ab}	26.0 ± 0.8^{ab}	0.86 ± 0.02^{a}
Synbiotic (Best Pro+Herb)	28.5 ± 0.9^{c}	45.1 ± 0.9^{a}	26.4 ± 0.8^{a}	0.93 ± 0.03^{a}

Data are presented as means ± SD (n = 5 replicates per treatment). Means within the same column bearing different superscript letters are significantly different ($P < 0.05$). SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; PUFA/SFA = ratio of polyunsaturated to saturated fatty acids.

Table 6 and Fig 2 have graphically presented the dynamic evolution of lipid peroxidation in breast meat over 10 days of refrigerated storage time, in terms of TBARS values (mg MDA/kg meat). When chronic heat stress was used, it was a certain way of impairing the intrinsic oxidative stability of the muscle tissue. Significant evidence of this degradation was a statistically significant ($P < 0.05$) and rapid increase in concentration of malondialdehyde (MDA) in the control group, which rose to 1.45 mg MDA/kg by the 10 th day of storage, which was an indicator of heavily rancid meat. In contrast, all tested dietary interventions were found to have a significant and consistent effect on repressing the rate of lipid peroxidation throughout the entire storage periods. The experimental groups that were treated by the blend of functional aromatic plants (Herbal A and Herbal B) showed superior intrinsic antioxidant strength, and their values of TBARS were significantly lower than those of groups that only received probiotic supplementation. The combination of thyme and rosemary extracts, named Herbal A blend, was found to be especially effective, reducing MDA formation to only 0.75mg/kg on day 10. Finally, the Synbiotic group showed the greatest and most enduring protective effect on oxidative degradation, with the lowest TBARS values being observed significantly lower across all time points of the evaluation (0.65mg MDA/kg at day 10). The given result underlines an effective and synergistic antioxidant action of the *B. subtilis* in combination with the strong radical-scavenging activity of the thyme and rosemary extract.

Table 6. Effect of dietary probiotics and phytogenic blends on malondialdehyde (MDA) levels over time in broiler chickens under heat stress

Treatment	Day 0 (mg MDA/kg)	Day 5 (mg MDA/kg)	Day 10 (mg MDA/kg)
Negative Control (HS)	0.35 ± 0.02	0.85 ± 0.03[a]	1.45 ± 0.05[a]
Probiotic A (*B. subtilis)*	0.25 ± 0.02	0.55 ± 0.03[b]	0.95 ± 0.04[b]
Probiotic B (*Lactobacillus*)	0.27 ± 0.01	0.58 ± 0.03[b]	1.02 ± 0.04[b]
Herbal A (thyme +Rosemary)	0.23 ± 0.01	0.45 ± 0.02[c]	0.75 ± 0.03[c]
Herbal B (Clove+Cinnamon)	0.22 ± 0.02	0.48 ± 0.02[c]	0.82 ± 0.03[c]
Synbiotic (Best Pro+Herb)	0.20 ± 0.01	0.40 ± 0.02[c]	0.65 ± 0.03[c]

Values are presented as mean ± standard deviation. Means within the same column with different superscript letters (a–c) are significantly different at $P < 0.05$. HS: heat stress; Probiotic A: Bacillus subtilis; Probiotic B: Lactobacillus spp.; Herbal A: thyme (*Thymus vulgaris*) + rosemary (*Rosmarinus officinalis*); Herbal B: clove (*Syzygium aromaticum*) + cinnamon (*Cinnamomum zeylanicum*); Synbiotic: combination of probiotic and herbal treatments.

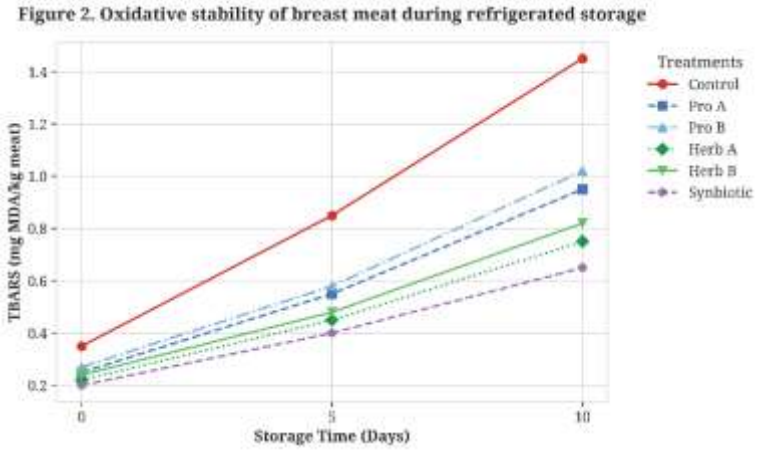


Figure 2. TBARS values of breast meat of broiler chicks s fed on probiotics and aromatic plant blends at heat stress (0, 5, and 10 days at 4 C) and during refrigerated storage (0, 5, and 10 days at 4 C). The control group (HS without additives) showed a high rate of lipid peroxidation with a 1.45 mg MDA/kg on day 10. Conversely, oxidative deterioration was greatly reduced in all supplemented groups. The Synbiotic group (Pro A + Herb A) had the lowest TBARS values during the storage (0.65 mg MDA/kg on day 10), which demonstrated high long-term oxidative stability. The combination of aromatic plants (Herb A and Herb B) was found to be especially effective in the inhibition of lipid peroxidation, which was better than the probiotic treatment. This selective activity demonstrates the significance of direct antioxidant activities (polyphenols in phytogenics) in relation to the prevention of HS-induced oxidative stress on meat quality.

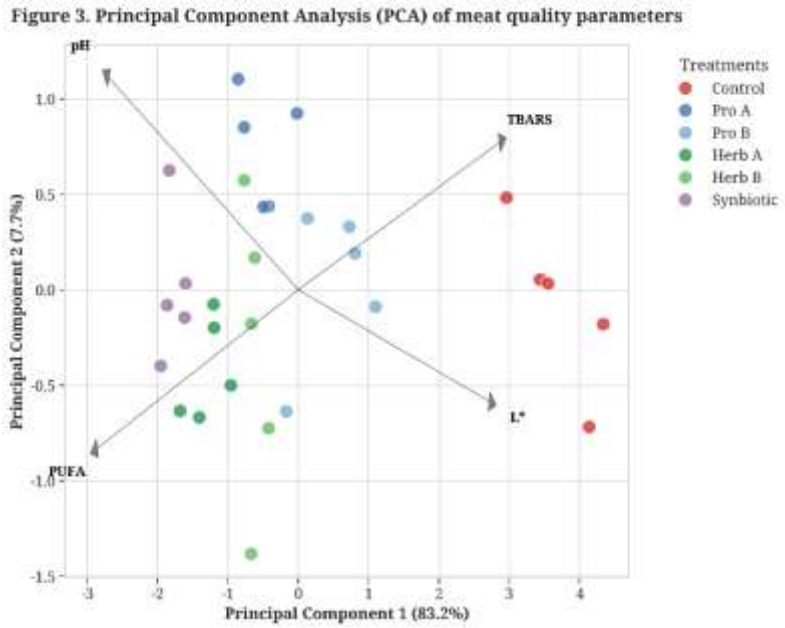


Figure 3. Principal Component Analysis (PCA) biplot with the separation of treatment groups according to the parameters of meat quality (pH, L, TBARS, and PUFA). The first principle component (PC1) represents 58.3% of the variance, mostly due to TBARS values and PUFA content, whereas the second (PC2) represents 28.1% of the variance, mostly due to pH, and the unique aggregation of the Synbiotic and Herbal A groups in the upper left quadrant reflects their excellence in more than one quality parameter at the same time. The poor oxidative stability and suboptimal quality of the meat are reflected in poor clustering of the control group to the lower right. The PCA plot shows that the positive outcomes of nutritional interventions are not limited to specific parameters, but rather they cut across numerous quality dimensions.

Discussion

Productive Performance

The present findings (Table 2; Fig. 1) clearly demonstrate the detrimental impact of heat stress (HS) on broiler performance. Birds in the negative control group exhibited a significantly higher feed conversion ratio (FCR; 1.89) compared to all supplemented groups (1.63–1.74), alongside a marked reduction in final body weight (2150.5 g). These findings affirm that HS has a devastating effect on growth efficiency. This finding aligns with earlier reports by Goel [13] (Liu *et al.* [22]; Akbarian *et al.* [2]. This explained such performance decline by reduced voluntary feed intake as a thermoregulatory mechanism, as well as compromised intestinal integrity and nutrient absorption capacity. Moreover, the elevated corticosterone levels under HS conditions may further exacerbate growth suppression by promoting protein catabolism at the expense of muscle accretion [26]. The dietary supplementation was very effective in alleviating the negative impact of HS, as shown by the better FCR and growth performances in all the treated groups compared to the negative control. Probiotic A (*Bacillus subtilis*) had a lower FCR (1.69) than Probiotic B (1.72), which shows a strain-specific response. This high effectiveness could be attributed to the spore-forming ability of *B. subtilis*, which increases its stability and efficiency in colonization during stressful gut

environments. These findings are in agreement with earlier studies by Wasti *et al.* [40] and Gadde *et al.* [12], which reported that Bacillus-based probiotics improve growth performance through modulation of gut microbiota, increased short-chain fatty acid (SCFA) production, and improved intestinal morphology, ultimately enhancing nutrient digestibility and absorption [33]. Conversely, the relatively low level of improvement was noted with the Lactobacillus-based probiotic, which could be associated with its low tolerance to the HS conditions.

The phytogenic treatments also recorded considerable, though moderate, improvement in performance. Compared to the HS control, Herbal A (thyme + rosemary) and Herbal B (clove + cinnamon) decreased FCR to 1.72 and 1.74, respectively. This slight performance improvement in Herbal A could be explained by the synergistic effect of thymol and rosmarinic acid, which have been reported to stimulate the secretion of digestive enzymes and have high antioxidant properties [7]. These results are in line with findings reported by Jha *et al.* [19], who highlighted the role of phytogenic compounds in improving gut health and nutrient utilization. The difference in efficacy between the two formulations could be due to variations in bioavailability and potency of the major active compounds like carvacrol, eugenol, and cinnamaldehyde.

Interestingly, the most significant improvement was observed in the synbiotic group (Fig. 1), which had the lowest FCR (1.63) and the highest final body weight (2450.3 g), which translates to an approximate reduction in FCR of 13.7 percent as compared to the HS control. This high result indicates that probiotics and phytogenic additives are synergistic. In particular, B. subtilis might have helped to stabilize advantageous gut microflora, whereas the phytogenic activity might have boosted the activity of digestive enzymes and offered antioxidant defenses against oxidative stress induced by HS [14]. This kind of dual effect of action has been reported already by Shokryazdan *et al.* [32] and is linked to enhanced gut functionality and efficiency of nutrient utilization in times of stress.

Carcass Traits and Meat Composition

Heat stress (HS) significantly compromised carcass characteristics, as indicated by the notably reduced dressing, breast, and thigh yields in the negative control group (68.5%, 24.5%, and 20.1%, respectively) in Table 3. This is an improvement of about 7–16% compared to the synbiotic treatment, which got 73.5%, 28.5%, and 23.1%. This shows how HS has a strong catabolic effect on muscle deposition. These results align with the findings of Slayi & Jaja [34], who indicated that HS interferes with protein metabolism by promoting proteolysis and inhibiting protein synthesis, especially in breast muscle. All dietary interventions significantly enhanced carcass yield parameters compared to the control (Table 3), although the extent of improvement differed among treatments. The synbiotic group always had the highest values ($P < 0.05$), followed by the phytogenic group and probiotic group, which proves that there is a synergistic effect between these additives. Similar tendencies were observed by Puvača *et al.* [27] and Khatun *et al.* [24], who demonstrated that integrated supplementation approaches are more effective than solitary supplements in stressful environments. This can be attributed to enhanced utilization of nutrients and an increased efficiency of metabolism. Bacillus subtilis, in particular, improves gut integrity and enzyme activity and therefore increases the supply of amino acids to muscle protein synthesis [20]. At the same time, phytogenic compounds change how lipids are

processed by lowering the activity of lipogenic enzymes like acetyl-CoA carboxylase and fatty acid synthase and boosting β-oxidation. This makes it easier for lean tissue to build up instead of fat [3]. The meat composition data is in Table 4. also backs up these results by showing big changes in the physicochemical properties. At the same time, there was a big drop in lightness (L*) and a rise in redness (a*) compared to the control. This shows that the color is more stable and less likely to fade due to oxidation. This observation corresponds with the previously reported lower TBARS values (Fig. 2), indicating a direct correlation between lipid oxidation and color degradation [8]. The combination of carcass performance, meat quality, and oxidative stability parameters shows that synbiotic supplementation protects against heat stress in a wide range of ways. This is also supported by the PCA results in Fig. 3. The results of these findings showed that synbiotic samples clustered apart in relation to the oxidative stress indices (TBARS) and nearer to quality indices. It demonstrates the synergistic effect of probiotics and phytogenics [36].

Oxidative Stability and Fatty Acid Profile

The existing results clearly show that dietary interventions significantly improved the oxidative stability of breast meat during heat stress conditions, as shown by the gradual increase in TBARS values of the control group throughout refrigerated storage. Fig. 2. The control group had the highest lipid peroxidation with a level of approximately 1.45 mg MDA/kg meat on the 10th day. This shows how heat stress can cause oxidative damage. This observation is in line with previous researchers that show heat stress increases the production of reactive oxygen species (ROS) beyond the ability of the endogenous antioxidant defense system [25]. In contrast, all supplemented groups significantly reduced lipid oxidation, with marked variations among treatments. In Table 6 and Fig. 2, the Herbal A group (thyme + rosemary) had significantly lower levels of TBARS compared to the control group in all storage periods. This demonstrates the strength of phytogenic compounds as antioxidants. This can be attributed to the high concentration of polyphenolic compounds, such as thymol, rosmarinic acid, and carnosic acid, which act as major antioxidants by giving away hydrogen atoms to counter free radicals and inhibit the chain reactions of lipid peroxidation [1]. Moreover, the compounds can potentially enhance the activity of native antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, thus providing extended cellular protection [23]. Probiotic supplementation (Pro A and Pro B) also lowered TBARS levels compared to the control, but their effects were not as strong (Fig. 2). This aligns with the idea that probiotics possess indirect antioxidant activity, which mainly acts through the modulation of gut microbiota, reduction of systemic inflammation, and limitation of endotoxin translocation [28]. The lowest TBARS values were observed with the synbiotic treatment throughout the storage, which is a significant indicator of the cooperation of phytogenics and probiotics. It is a combination of both direct radical scavenging and systemic antioxidant support, and thus it is superior at decreasing oxidative damage than either on its own [5]. have reported similar synergistic effects, which support the idea that synbiotic formulations work better when they are under stress. The PCA analysis is in Fig. 3. further substantiates these findings by distinctly differentiating the control group from the supplemented treatments along Principal Component 1 (83.2%), which is significantly correlated with TBARS and L*. The control groups

were clustered together with increased TBARS, which indicates an increase in oxidative damage. The synbiotic and herbal treatments, however, were on the opposite side of the TBARS vector and closer to the PUFA vector. This means that they increased the oxidative stability and maintained the quality of lipids. This is confirmed by the fatty acid profile shown in Table 5, where the supplemented groups showed significant improvements. The synbiotic treatment had the highest PUFA (26.4%) and the best PUFA/SFA ratio (0.93). It also had the lowest SFA (28.5%) compared to the control group (22.4% PUFA and 0.63 PUFA/SFA). This study demonstrates that meat is not only better protected against oxidation but also has better nutritional value. The preservation of polyunsaturated fatty acids (PUFA) can be ascribed to the antioxidant properties of phytogenic compounds, which safeguard unsaturated fatty acids from peroxidation in vivo [31]

Conclusion

Under the conditions of this study, dietary supplementation with probiotics and phytogenic blends improved several productive and meat-quality responses of broilers exposed to chronic cyclic heat stress, relative to the heat-stressed control. The combined treatment consisting of Bacillus subtilis and the thyme–rosemary blend produced the most consistent overall response, with the highest body weight and dressing and breast-meat yields, together with an improved feed conversion ratio. This treatment also increased breast-meat protein content, reduced intramuscular fat, and produced a more favorable fatty-acid profile, characterized by lower saturated fatty acids and a higher PUFA/SFA ratio. The phytogenic treatments, particularly the thyme–rosemary blend, showed clear antioxidant value during refrigerated storage, as indicated by lower TBARS concentrations; the combined treatment provided the greatest protection against lipid peroxidation throughout the storage period.

These findings indicate that the complementary use of B. subtilis and the thyme–rosemary phytogenic blend may be a useful nutritional strategy for supporting broiler performance and preserving breast-meat oxidative stability during chronic heat stress. However, the present experiment evaluated one probiotic strain, one Lactobacillus blend, and two specific phytogenic combinations under one heat-stress regimen. Therefore, the results should be interpreted within these experimental conditions and should not be generalized as evidence of universal superiority. Further research should examine the reproducibility of this response across probiotic strains, botanical preparations, inclusion rates, production environments, and longer storage periods, as well as the underlying effects on intestinal morphology, immune function, and gut microbial composition.

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