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Research Article

Effects of Feeding Thyme, Rosemary, and Oregano Powder Supplementation on Broiler Chickens: Performance, Meat Quality, Hematology and Serum Biochemistry

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Abstract | The study examined the impact of thyme, rosemary, and oregano powder on growth performance, meat quality, hematology, serum chemistry, and profitability in commercial broiler chickens. Four feeding treatments were randomly assigned to 480-day-old Indian River Meat chicks, with six replicates, each consisting of 20 birds. The control group (T_1) received a basal diet, while the T_2, T_3, and T_4 groups received thyme, rosemary, and oregano powder supplementation at 5 g/kg of feed, respectively. A corn soybean meal-based mash diet is supplied in two phases (starter phase 0-21 days; ME = 2958 kcal/kg, CP = 21.77%, and grower phase 22-35 days; ME = 3046 kcal/kg, CP = 20.31%). The findings indicated that growth performance showed no significant differences among the dietary groups. However, rosemary (FBW= 1613.1g/b, BWG=1571.39 g/b, FI= 2469.18 g/b, FCR=1.56) and oregano (FBW= 1589.89 g/b, BWG=1548.11g/b, FI= 2458.73 g/b, FCR=1.59) supplemented groups showed the highest final body weight, body weight gain, FI and better FCR. Meat quality data represents that rosemary group had significantly (P<0.05) higher water-holding capacity than other two groups and control group. Meat color data showed that control group had the lowest lightness and redness values whereas thyme and oregano group had the highest values. The yellowness data represented the maximum values found in the oregano supplemented group and minimum in the control group, and they were significantly (P<0.05) differed from the control group. The rosemary group had the highest significant (P<0.05) levels of hemoglobin, total erythrocyte counts, and packed cell volume as compared to control and other two groups. Thyme group showed significantly (P<0.05) lower levels of total cholesterol (86.97 mg/dl), triglyceride (75.89 mg/dl) and low-density lipoprotein (37.39 mg/dl) but higher in high density lipo-protein (61.43 mg/dl). Aspartate aminotransferase enzyme levels were significantly (P<0.05) higher in the control group but lower in the oregano group. The herbs supplemented groups had a better benefit-cost ratio. It was summarised that the potential use of powder extract of herbs in broiler diet improved growth performance, meat quality and reduced cholesterol and triglyceride levels by maintaining good liver condition. So, this powder of herbs may be used as an alternative to antibiotic growth promoters.

Keywords | Growth performance, Meat quality, Hematology, Lipid profiles, Liver enzymes, Profitability

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INTRODUCTION

Over the years, intensive farming practices have been effectively employed to satisfy the rising demand for chicken products. Nevertheless, this approach also poses some serious challenges to the well-being of poultry, such as digestive dysfunctions and physiological stresses, which adversely affect the overall quality of poultry products and the profits of the producers (Averos and Estevez, 2018). Previously, antibiotic growth promoters (AGPs) have been widely used in chicken production for a variety of reasons to improve growth performance, feed conversion ratio (FCR), intestinal health, stress resistance, disease prevention and treatment, and animal health. However, the consumers were concerned about the use of antibiotics as therapeutics and for growth promotion could lead to a problem (Butaye *et al.*, 2003), such as the rise of antibiotic-resistant pathogenic microorganisms and the presence of antibiotic residues in chicken products (Yang *et al.*, 2009). Consequently, numerous nations, including the European Union (EU), Japan, Australia, Bangladesh etc. have prohibited the use and marketing of AGPs, restricting their application solely to essential therapeutic purposes (Windisch *et al.*, 2008).

Herbs are plant-based feed supplements has drawn interest from researchers and is gaining traction as an alternative to AGPs. Out of all the plant-derived feed supplements, three major plant supplements from the Lamiaceae family—thyme (*Thymus vulgaris* L.), rosemary (*Rosmarinus officinalis* L.), and oregano (*Origanum vulgare* L.)—are broadly using in poultry production. Thyme, a well-known perennial aromatic herbaceous plant, is one of the world's most extensively used herbs for both culinary applications as a spice for distinct flavour and medicinal purposes. In poultry nutrition, thyme is utilised as an herbal feed supplement, and thyme powder is derived from drying its leaves, stems, and flowers. Thymol (5-methyl-1-2-isopropyl phenol) and carvacrol (5-isoprophyl-1-2-isopropyl phenol), are the main components of herbs. Rosemary officinalis L., a naturally occurring aromatic herb, is widely known for having a high antioxidant content that enhances bird's performance (Kedir *et al.*, 2023). Botsoglou *et al.* (2005) stated that a few phenolic compounds, such as methylcarnosic acid, ursolic acid, carnosic acid, rosmanol, carnosol, and rosmarinic acid, are present in rosemary powder, which is produced by drying leaves. The presence of active chemical component (*Origanum vulgare* L.) in the aromatic herb oregano is well established (Ri *et al.*, 2017; Ghanima *et al.*, 2020).

Thyme, rosemary, and oregano have strong anti-inflammatory, antibacterial, and antioxidant qualities that have positive impacts on the physiology and immunity of the birds (Nieto *et al.*, 2010; Hasan and Awad, 2017; Tzora *et al.*, 2017; Alagawany *et al.*, 2018; Bauer *et al.*, 2019). Thymol have shown positive effects on broiler body weight, body weight gain, and feed utilisation, which ultimately results in increased economic benefits (Ali, 2014; Hasan and Awad, 2017). More precisely, rosemary may help broiler chickens' growth by boosting appetite and feed intake, enhancing digestion and absorption, encouraging saliva production, and synthesising bile acids that improve the birds' immune system, growth performance, and feed conversion ratio (Loestsher *et al.*, 2013; Mohammadi *et al.*, 2019). Alagawany *et al.* (2018) showed that adding oregano to chicken diets may increase feed intake and improve feed efficiency, feed digestion, reduce disease incidence, and increase productivity.

Although numerous published studies have demonstrated the positive impacts of herbs, in the Bangladeshi perspective, the multipurpose qualities of herbs on broiler chickens' physiological indicators, meat quality, and production performance are a new dimension. The present study therefore hypothesised that the powder of these herbs could enhance broiler growth performance, meat quality, blood hematological, lipid profiles, and liver enzymes.

Therefore, this study evaluates the impact of feeding thyme, rosemary, and oregano on broiler chickens' performance, meat quality, and blood biochemical profiles, contributing to the search for AGP alternatives in poultry nutrition.

MATERIALS AND METHODS

Animal Ethics Approval

The methodological approaches like humane handling, euthanasia etc. of the birds were approved by the Bangladesh Agricultural University's Animal Welfare and Experimentation Ethics Committee (AWEEC), Mymensingh, Bangladesh (Approval number: AWEEC/BAU/2024[22]).

Site and Experimental Birds

For the experimental trial, a total of 480 one-day-old Indian River Meat (IR) commercial broiler chicks were purchased from C.P. Bangladesh commercial hatchery. The C. P. is in a leading position in Bangladesh for their research and development as well as producing high-quality chicks' and poultry and fish feeds. The feeding trial lasted 35 days at the Bangladesh Agricultural University Poultry Farm in Mymensingh. The chicks were divided among four dietary feeding regimens: control, thyme, rosemary and oregano groups. Each group had six replicates, and each replicated had twenty birds. The experimental design was completely randomized design (CRD). The randomisation was done by using a random number generator to distribute treatments and replication into twenty-four experimental units.

Experimental Diet and Management

A corn-soybean meal-based mash diet was formulated

in accordance with the IR commercial broiler chicken management guide to satisfy the birds' nutritional needs (Aviagen, 2022). There were four different diets for the birds. The first group of chicks received Diet 1: Basal diet (control diet), and the remaining groups were named as Diet 2-4: basal diet supplemented with thyme, rosemary, and oregano @ 5 g/kg feed, respectively. Birds were fed the prepared diets in two phases: starter phase (0–21 days) and grower phase (22–35 days).

Table 1: Ingredients and nutrient composition of starter and grower diets (kg/100 kg).

Ingredients (%)	Starter diet (0-21 days)	Grower diet (22-35 days)
Corn	50.93	51.1
Soybean meal	36.0	34.0
Soybean oil	4.0	5.0
Rice polish	4.0	5.0
Limestone	1.40	1.50
Sodium chloride (NaCl)	0.32	0.35
Di calcium phosphate	1.50	1.50
L-Lysine	0.60	0.50
DL-Methionine	0.50	0.40
Choline chloride	0.10	0.10
*Broiler premix	0.25	0.25
Analyzed nutrients (%)		
ME (kcal/kg)	2958	3046
Crude protein	21.77	20.31
Crude fat	9.10	10.0
Crude fiber	3.90	4.00
Calcium	1.10	1.11
Phosphorus	0.46	0.52

*Broiler premix contained vitamin A: 12.50 MIU; vitamin D: 2.50 MIU; vitamin E: 25 g; vitamin K: 4 g; Iron: 24 g; Zinc: 40 g; Manganese: 48 g; Selenium: 0.12 g; Cobalt: 0.30 g. **ME:** metabolizable energy, **%:** percentage, **kg:** kilogram.

Table 1 shows the ingredients and the nutrient composition of the basal diet (ME=2958 and 3046 Kcal/kg, CP=21.77 and 20.31%, Ca=1.10 and 1.11%, P=0.46 and 0.52%) that satisfied the nutritional requirements of the IR commercial broiler strain. An open-sided house was partitioned with wire mash and bamboo into 24 equal-sized pens in which the experimental birds were randomly allocated according to treatment and replication. All management procedures were followed by the IR commercial broiler chicken management guide (Aviagen, 2022). Feed and water were supplied to the birds three times daily on an *ad libitum* basis. Feeders and drinkers were cleaned on regular basis and lighting was maintained according to birds' age. Biosecurity protocols were strictly followed during the trial, and it was restricted for visitors. A foot bath with a disinfection solution was kept up at the shed's gate. Using an automatic thermo-hygrometer, the daily room temperature and humidity were noted in the daily activities sheet (HTC-2, Velveeta, Makkar Trading Company, India). The temperature and humidity were recorded three times daily (6:00 am, 12:00 pm, 6:00 pm).

Record Keeping and Sampling

Every seven days interval, the body weight (BW) and feed intake (FI) of broiler chickens were noted. Every day, the mortality was recorded and FCR was calculated based on BWG and FI. On the final day of the experiment, six birds, one from each replication group, were randomly selected according to their average weight and subsequently euthanized by decapitation. Before decapitation of the birds, a sharp equipment and place was properly selected and restrained the birds. To sever the head from the body, birds were positioned properly by extending the neck fully. A single and soft motion blade was used to sever the head. After confirming death, blood and breast meat samples were collected for further analysis. Two types of plastic tubes were used for haematology and serum biochemistry determination. Ethylenediamine tetra acetic acid (EDTA) with anticoagulant containers was used for hematological assessment, and without anticoagulant containers were used for estimating serum profiles.

Meat Quality Analysis

Water holding capacity (WHC%), cooking loss (CL%), drip loss (DL%), and meat lightness (L*), redness (a*), and yellowness (b*) all were included in the examination of meat quality. Centrifugation assay was used to measure the WHC of breast meat. About 1g breast meat sample weighed with the help of digital weighing balance, cut into cubes from each breast meat sample, kept in a centrifuge tube, and then centrifuged at 10000 RCF at 4°C for 10 minutes by using a centrifuge machine (ScanSpeed 1730R Microcentrifuge, LaboGene, Denmark). The WHC was determined by the method of Zhang *et al.* (1995). To determine the CL, the samples were weighed with a digital weighing balance, put into a polythene bag, and cooked in a water bath at 80°C for 30 minutes. After that, the samples were removed from the water bath and allowed to stand at room temperature for 5 minutes (Lylon *et al.*, 2005). They were then weighed again, and the CL was determined by dividing the cooked sample weight by the precooked sample weight:

$$CL(\%) = \frac{Initial\ weight - Cooked\ weight}{Initial\ weight} \times 100$$

According to Benli (2015), to calculate DL of the breast meat, the samples were weighed using a digital weighing balance and put in a pot separately to let the meat juice drain out, making sure the sample did not touch the drain-

ing juice. After that the samples were then stored in a refrigerator at -20°C for 24 hours, then reweighed, and DL was calculated from the following formula:

$$DL(\%) = \frac{Initial\ weight - Chilled\ weight}{Initial\ weight} \times 100$$

Color space values of L* (lightness), a* (redness), and b* (yellowness) were assessed using the Konica Minolta Chroma Metre CR-410 (Konica Minolta Inc., Tokyo, Japan) at 24 hours after sampling. Prior to usage, the apparatus was calibrated using a white plate (Y = 86.5, X = 0.3166, and Y = 0.3242). Before cooking, three random readings were made on the outside of the t skinless chicken breast samples (Benli, 2015).

Hematology and Serum Biochemistry Determination

Following the normal protocol, the following hematological parameters were estimated within two hours of the blood collection: hemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), neutrophil, eosinophil, lymphocyte, and monocyte (Ghai, 2012). After blood coagulation, the serum was separated by centrifugation at 4°C for 10 minutes at 3000 rpm, and tubes were then stored in a freezer at -20°C. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) were determined by the spectrophotometric method using T80 Spectrometer (USA) and low-density lipoprotein (LDL) was estimated according to Bartl *et al.* (1983). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were evaluated according to the method of Reitman and Frankel (1957).

Cost-Benefit Analysis

In poultry farming, feed costs account for 60–70% of total production costs (EI Deek *et al.*, 2020). Cost-benefit analysis is essential for evaluating the cost-effectiveness of various feed formulations, additives, or substitute feed ingredients. It's also crucial for striking a balance between dietary expenses and performance metrics like growth rate, FCR and meat quality. Therefore, cost-benefit analysis helps producers make data-driven decisions that maximise sustainability, profitability, and production. Cost benefit analysis was calculated by identifying the total cost (chick, feed and labor etc.) and benefit (sales of broiler) from the broiler production. The formula was: Net profit=Total benefits–total costs. After that, benefit cost ratio was determined by the following formula: Benefit-Cost Ratio (BCR)=Total costs/total benefits. However, the total production cost per bird and per kg broiler and profit earned were calculated. The additional costs incurred for test ingredients were also considered in performing cost benefit analysis.

Statistical Analysis

The SAS software was used to statistically determine all the datasets gathered from the current experiment using a one-way ANOVA (SAS, 2009). The mean values for each treatment group were compared using Duncan's Multiple Range Test (DMRT) because DMRT is popular in agricultural research for ranking the treatments, and that helped to identify which means are statistically comparable or different with each other and denoted by distinct letters (for example, a, b and c).

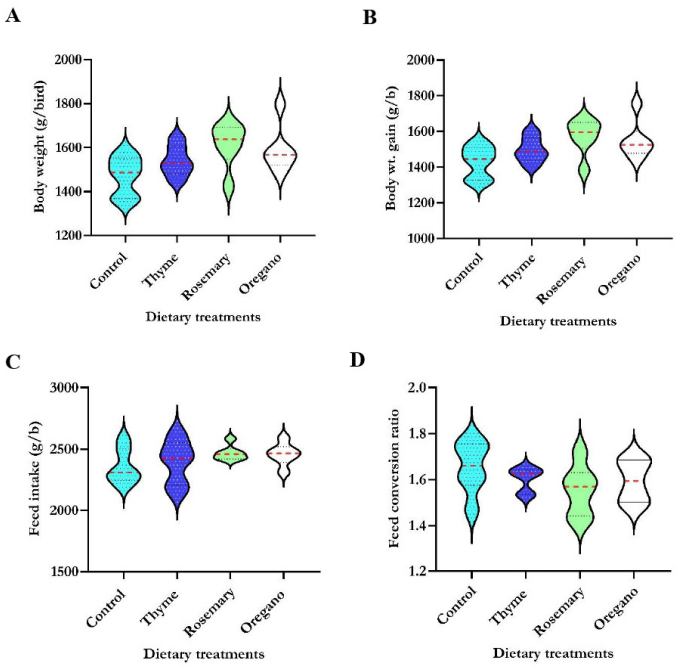

Figure 1: The violin plots show the final body weight(A), body weight gain (B), feed intake (C) and feed conversion ratio (D) of control and other supplemented groups (thyme (5g/kg), rosemary (5g/kg) and oregano (5g/kg)). The box represents the 95% confidence interval of the data, and the horizontal red line represents the mean values. Though they vary numerically, none of the growth parameters did not differs significantly from the others.

RESULTS

Growth Performance

An overview of feeding thyme, rosemary and oregano powder on the growth performance of broiler chickens was shown in Figure 1. The growth performance data did not show substantial statistical difference among the treatment groups, despite a trend of numerical variance. In contrast to the control and other groups, the rosemary-supplemented group had the highest body weight (1613.09 g/b) and feed intake (2824.26 g/b). The rosemary supplemented group showed the best improved FCR as compared to other supplemented and control groups. In some context, non-significant result is important because statistically

non-significant results support the null hypothesis. In this experiment, while the inclusion of herbs in the diets did not significantly affect growth rate, feed intake, or feed conversion ratio, however it may reduce some unnecessary expenses. The reasons for the non-significant results of these data remain unclear; nevertheless, it may be possible that inappropriate or excessive herb supplementation, together with adverse interactions with the basal diet or physiological responses in the birds, may be contributing factors.

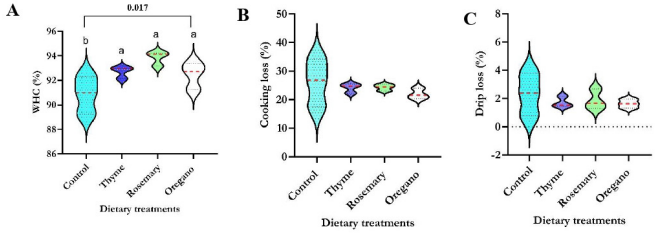

Figure 2: The violin plots illustrate the water holding capacity (A), cooking loss (B) and drip loss (C) of broiler chicken meat quality. Four dietary groups such as control group, thyme (5g/kg), rosemary (5g/kg) and oregano (5g/kg) supplemented group. The box represents the 95% confidence interval of the data, and the horizontal red line represents the mean values. a.b within a column, exhibit the stated level of significant differences (P-0.017).

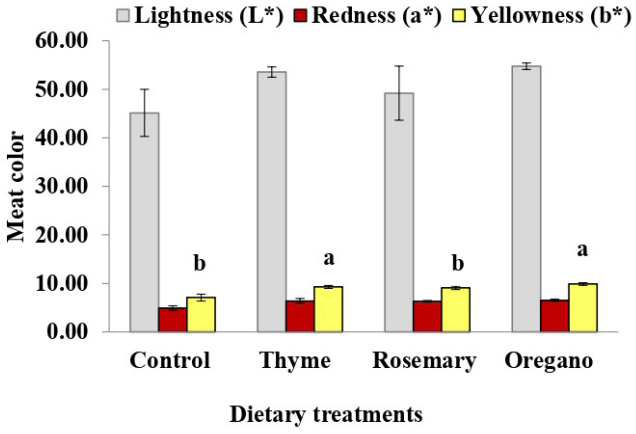

Figure 3: The bar graph indicates the three different colors (lightness, redness and yellowness) of breast meat of broiler chickens. There were four dietary groups: control, thyme (5g/kg), rosemary (5g/kg), and oregano (5g/kg). a, b values with distinct alphabetic superscripts within a column exhibit the stated level of significant differences (P=0.003). Bar indicates the standard error of mean.

Breast Meat Quality

The impact of thyme, rosemary, and oregano on the WHC, CL, and DL percentage of breast meat is displayed in Figure 2. Interestingly, the rosemary-supplemented group had the greatest WHC ($P < 0.05$) compared to the control group. In contrast to the treated groups, the control group showed the maximum values for DL and CL. Regarding meat colour, neither the control nor treatment groups had a significant impact on the breast muscle's lightness (L*) or redness (a*). However, the thyme (lightness: 54.56, redness: 6.33) and oregano (lightness: 54.75, redness:6.57) groups had the highest lightness and redness values. The thyme and oregano-supplemented groups, however, had a substantial ($P < 0.05$) impact on the yellowness (b*) value when compared to the control group (Figure 3). Herb supplements can improve meat flavours and aroma, tenderness and juiciness by reducing oxidative damage of the meat. Herbs supplemented diet convert feed into organic meat as well as leaner meat that appeals to health-conscious consumers. Broiler meat's lightness, redness, and yellowness are not only the aesthetic parameters but also essential features of nutritional quality, freshness indicators, processing impact, storage and shelf life, and consumer preference.

Hematology Study

Table 2 shows the hematological characteristics of broiler chickens treated with oregano, rosemary and thyme powder. There were significant differences ($P<0.05$) in Hb, PCV, and TEC across the treated groups. Specifically, the rosemary-supplemented group exhibited the highest Hb, TEC and PCV levels, followed by the oregano, thyme and control groups. The physiological condition, health, and production of broiler chickens are intimately related to hematological alterations. High levels of TEC, Hb, and PCV indicate dehydration due to high temperature, and lower levels of these parameters signify anaemia, poor nutrition, or parasitic infections.

Table 2: Effects of feeding thyme, rosemary, and oregano powder supplementation on the hematology of broiler chickens.

Parameters	Treatments[1]				SEM	P-value
	T_1	T_2	T_3	T_4		
Hemoglobin	12.40^{c}	13.53^{b}	14.93^{a}	14.27^{ab}	0.30	0.001
TEC	231.33^{b}	249.33^{a}	265.67^{a}	251.00^{a}	4.33	0.013
PCV	19.33^{b}	22.33^{a}	23.67^{a}	22.67^{a}	0.67	0.030
ESR	5.17	6.67	5.71	6.33	0.31	0.345
Neutrophil	30.00	31.33	32.67	30.67	0.78	0.719
Eosinophil	2.67	3.67	4.33	4.00	0.43	0.620
Lymphocyte	61.33	64.67	65.67	64.67	0.88	0.365
Monocyte	1.22	2.04	1.67	1.98	0.15	0.205

a, b, and c values with distinct alphabetic superscripts within a row exhibit significant differences ($P <0.05$). The control group is denoted by T_1, whereas the thyme (5g/kg), rosemary (5g/kg) and oregano (5g/kg) powder supplementation groups are T_2, T_3 and T_4; **TEC:** Total erythrocyte count; **PCV:** Packed cell volume; **ESR:** Erythrocyte sedimentation rate.

Serum Biochemistry Assays

The results of supplementing thyme, rosemary, and oregano powder on the lipid profiles and liver enzymes of broiler chickens are displayed in Figure 4 and Figure 5, respectively. The data on lipid profiles showed significant variability in cholesterol levels among the treatment groups ($P<0.05$), with the control group exhibiting the highest cholesterol level (106.54 mg/dl), as compared to all other supplemented groups (thyme: 86.97 mg/dl, rosemary: 88.80 mg/dl, and oregano: 88.50 mg/dl, respectively). However, the levels of HDL and TG did not statistically different ($P>0.05$) across the treated groups. Oregano supplemented group had the lowest (36.72 mg/dl) LDL values as compared to the other two groups (thyme: 37.39 mg/dl, rosemary 38.19 mg/dl) and control group (46.78 mg/dl) which also revealed statistical variation ($P<0.05$).

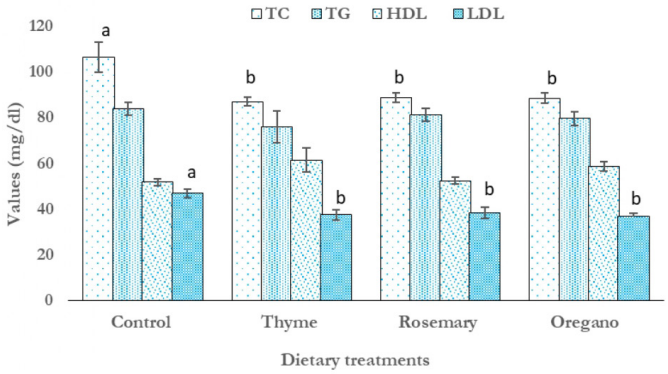

Figure 4: The bar graph shows the effects of feeding thyme, rosemary, and oregano powder on the lipid profiles of broiler chickens. There were four treatment groups like control, thyme (5g/kg), rosemary (5g/kg), and oregano (5g/kg) groups. ab within a column, values with different alphabetic superscripts differ significantly ($P<0.05$). Here, TC-Total cholesterol, TG-Triglyceride, HDL-High density lipoprotein, LDL-Low density lipo-protein. mg/dl-milligrams per deciliter. Bar indicates the standard error of mean.

Regarding liver enzyme functions, there were notable ($P<0.05$) variations in serum aspartate aminotransferase (AST) levels among the supplemented groups. The highest AST data were recorded in the control group (13.97 U/L), and the minimum level was found in rosemary (6.71 U/L) and oregano-supplemented groups (4.27 U/L). On the other hand, there were no notable ($P > 0.05$) variations found in the serum levels of alanine aminotransferase (ALT) across the treatment groups. For evaluating the liver health and general metabolic function of broiler chickens, AST and ALT trends are extremely important. These enzymes are essential for the metabolism of amino acids and released into the bloodstream when liver cells are harmed or under stress. Elevated levels of AST and ALT suggest liver dysfunction or hepatocellular damage.

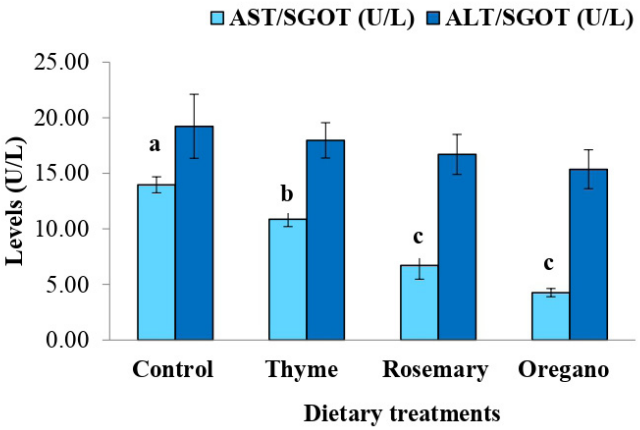

Figure 5: The bar graph demonstrates the effects of feeding thyme, rosemary, and oregano powder on the liver enzymes of broiler chickens. There were four different dietary groups. be Within a column, values with different alphabetic superscripts differ significantly ($P<0.001$). Here, AST-Aspartate amino transferase, ALT-Alanine amino transferase. U/L= units per liter.

Cost-Benefit Analysis

Table 3 shows the cost-benefit analyses of broiler chickens receiving dietary supplementation of thyme, rosemary, and oregano powder. Feed cost, total cost per live bird, total cost per kg of live bird, total income per live bird, total gross profit per live bird, total gross profit per kg of live bird, and cost-benefit ratio (BCR) did not show any significant variations. In comparison to the control group, a numerical improvement was found in the herb-supplemented groups. The rosemary added group had a higher profit margin and BCR than the oregano and thyme groups, while the control group had the lowest profit margin. A key component of lucrative and sustainable farming is the cost-benefit trends in the production of broiler chickens. Industry needs cost-benefit trends for profit maximisation, and industry can offer farmers to adopt practices that directly enhance profitability while minimising input costs. Cost-benefit analyses demonstrate the financial benefits of improved FCR achieved by better management and nutrition techniques. Since feed accounts for 60–70% of production expenses, the industry may focus minimising waste and optimising growth.

DISCUSSION

Growth Performance

The present study revealed no statistically significant changes in growth performance when the broiler diets were supplemented with thyme, rosemary, and oregano powder. There were some limitations of the study, including a small sample size, short duration of the feeding trial, and the quality of the herbs used, which altogether could result in insignificant growth performance. However, there was a noticeable numerical trend indicating a considerable

Table 3: Thyme, rosemary, and oregano powder supplementation on the benefit cost ratio of broiler chickens.

Items		Treatments[1]				SEM	P Value
		T_1	T_2	T_3	T_4		
Body weight	Body weight (kg/bird)	1.47	1.54	1.61	1.59	0.02	0.067
Feed	Feed intake (kg)/bird)	2.36	2.40	2.47	2.46	0.03	0.405
	Basal feed price BDT (kg)	61.00	61.00	61.00	61.00	-	-
	Feed cost BDT (kg/bird)	143.96	146.40	150.67	150.06	1.58	0.394
Thyme, Rosemary, Oregano	Amount consumed (g)	0.00	14.05	14.12	14.07	-	-
	Supplementation price, BDT/g	0.00	1.20	1.27	0.95	-	-
	Supplementation cost (BDT/bird)	0.00	16.86	17.93	13.37	-	-
Others (chicks, vaccine, litter, electricity, disinfectant, labor cost, transport etc.)		60.00	60.00	60.00	60.00	-	-
Total cost, BDT/bird		203.96	206.40	210.67	210.06	1.59	0.377
Total cost, BDT/kg live bird		138.75	134.03	130.85	132.11	1.52	0.321
Sale price	Price (BDT)/kg live bird	160.00	160.00	160.00	160.00	-	-
	Total income (BDT)/live bird	235.20	246.04	257.60	254.40	3.37	0.073
Gross profit (BDT/live bird)		31.24	38.64	46.93	44.34	2.82	0.229
Gross profit (BDT/kg live bird)		21.25	25.09	29.14	27.89	1.69	0.321
Benefit cost ratio (BCR)		1.47	1.54	1.61	1.58	1.19	0.369

[1]T_1 represents the control group; T_2, T_3 and T_4 groups represent the supplementation of thyme (5g/kg), rosemary (5g/kg) and oregano (5g/kg) powder, respectively; **g:** gram; **kg:** kilogram; **BDT:** Bangladeshi taka.

improvement in the growth performance, such as FBW, BWG, FI, and FCR, in rosemary supplemented group. The superior performance of rosemary compared to thyme and oregano in broiler chickens could be attributed to its richer antioxidant profile (rosmarinic and carnosic acids), antimicrobial efficacy, synergistic effect with diet, dose response relationship may align better with the broiler physiological needs and variation in chemical composition etc. Its antibacterial qualities enhance gut health by lowering harmful bacteria and increasing helpful microbial populations. Additionally, rosemary supplementation has been linked to increased nutrient absorption, enhanced appetite, improved digestion of bile acids, along with absorption and synthesis of lipids, and secretion of salivary enzymes (Christaki *et al*., 2011). This would improve the nutrient absorption and utilisation in the gut of broiler chickens ultimately resulting in the enhanced growth and feed efficiency observed in the broilers (Jamroz *et al*., 2006). The present findings align with several previous studies reporting improved BW, BWG, FI, and FCR in broilers supplemented with thyme, rosemary, and oregano. According to Hasan and Awad (2017) and Tayeb *et al*. (2019), broilers fed 5 g/kg thyme powder exhibited improved FBW and BWG. Similarly, Ampode and Mendoza (2022) reported that 3% or 5% oregano powder supplementation improved birds' performance, as compared to the control group, while Ghazalah and Ali (2008) and Tayeb *et al*. (2019) noted improvements in growth performance with 5 g/kg rosemary powder.

The incorporation of thyme, rosemary and oregano powder into the broiler chickens' diet, increased FI. This aligns with prior findings where researchers found that feed consumption is increased in broiler chickens that received these types of herbs in their diet (Ghazalah and Ali, 2008; Hasan and Awad, 2017; Ampode and Mendoza, 2022). Thyme has been shown to be a possible substitute for AGPs that can also improve the FI in broiler when supplemented at 5 g/kg feed (Fallah and Mirzaei, 2016). Similarly, rosemary powder at 5 g/kg (Tayeb *et al*., 2019) and oregano powder at 150 mg/kg (Ri *et al*., 2017) increased FI in broiler birds in contrast to the control group. Herb contains bioactive components that are endowed with antimicrobial and antioxidative properties that increase feed palatability, boost appetite and digestive enzyme secretion, and promote intestinal absorption in broilers that promote better growth (Windisch *et al*., 2008; Yang *et al*., 2009).

On the contrary, some studies reported that these herbs had no effect or having negative effect on broiler growth (Kamali Sangani *et al*., 2014; Soltani *et al*., 2016; Criste *et al*., 2017; Hasan and Awad, 2017; Ri *et al*., 2017; Tayeb *et al*., 2019). There were some reasons, like dosages, antinutritional factors, herb quality, unfavourable interactions, or physiological responses in the birds, that may be caused negative effects (Hong *et al*., 2012; Attia, 2018). The way that the basal diet and herb supplements combine can have a big impact on how well broiler chickens grow. These interactions depend on the bioactive

compounds in the herbs (e.g., antioxidants, antimicrobials, and growth-promoting agents) and ingredient and nutrient composition of the basal diet. A basal diet is high in fats or complex carbohydrates that improve nutrient breakdown and absorption, and herbs stimulate digestive secretions like bile and gastric enzymes. Supplementing basal diet with herbs can promote growth and meat quality, maintain cellular integrity by stopping lipid peroxidation.

Breast Meat Quality

Nowadays, meat scientists and chicken farmers alike are very concerned about meat quality, particularly when it comes to reaching targeted meat production levels (Mir *et al*., 2017). Rosemary treated group increased WHC of broiler meat, is a crucial characteristic for meat's texture, juiciness, and general acceptability among consumers (Park *et al*., 2015). Carnosic acid, rosmarinic acid, and flavonoids are abundant in rosemary powder and these antioxidants help to reduce oxidative stress in muscle tissues during slaughter and postmortem processing. Rosemary powder improves WHC by reducing inflammation, which can stop the breakdown of muscle proteins and the loss of water from muscle cells. Some herbs may influence the makeup of muscle fibres, encouraging the growth of more Type I (slow twitch) fibres, which are linked to improved WHC by improving its capacity to retain water.

The findings of this present study made it very clear that herbs have significant impact on yellowness of meat. Both thyme and oregano contain carotenoids such as lutein, zeaxanthin and β-carotene, which are plant pigments responsible for yellow, orange, and red coloration. These carotenoids are absorbed through the digestive system and are transported to various tissues, including muscles and fat, where they are deposited. The yellow pigmentation resulting from carotenoids increases the yellowness (b)* value in the meat. In addition, carotenoids are fat-soluble and stored in adipose tissues. More carotenoids are concentrated in muscle tissue due to increased fat deposition in the muscles, which makes the meat more noticeably yellow. The degree of yellowness in broiler chicken meat can affect consumer preferences in either a positive or negative way, depending on regional, cultural, and psychological factors. To improve market acceptance and match meat colour with consumer expectations, producers must carefully control nutritional formulas and marketing tactics. Yesilberg *et al*. (2011) reported that incorporating rosemary plants into diets lowered meat pH, prolonging the period before microbial degeneration and thereby enhancing meat quality. Park *et al*. (2015) noted significantly lower CL in ducks due to dried oregano powder supplementation, although no significant differences in pH were observed. In contrast, oregano powder supplementation in broiler chickens' diet did not get any notable variance in CL, DL, pH, lightness, yellowness, and redness of the breast meat (Ri *et al*., 2017). Similarly, Tayeb *et al*. (2019) found no significant differences in the meat's lightness, redness, or yellowness among broilers given thyme and rosemary. The use of thyme, rosemary, and oregano supplementation in broiler diets can arise from a variety of factors. These herbs may be included in broiler diets either through feed or as additives in drinking water, as well as their delivery method that can also influence the birds' metabolism and absorption. In other words, the quality of the could lead to variations in outcomes. Not only that, high temperatures or stress from poor ventilation might amplify or negate the positive effects of herbs, making results inconsistent across studies with different environmental conditions (Ri *et al*., 2017). These factors can influence the results regarding growth performance, meat quality, and other physiological outcomes in broilers. Thyme, rosemary, and oregano might help prevent oxidative stress and lipid peroxidation in meat, potentially enhancing its quality because of their antioxidant properties. The inappropriate dose of herbs may have a noticeable impact on meat quality. Fresh oregano, rosemary, and thyme have greater levels of bioactive substances and essential oils. Since fresh herbs' natural preservatives can be more effectively absorbed into the broiler's system, they may have a greater impact on the quality of the meat. Volatile oils are reduced during the drying process that's why dried herbs may lose part of their strength even though they are still beneficial. Dried herbs can still enhance meat texture and shelf life while retaining their antioxidant qualities.

Hematology Study

Hematological parameters serve as crucial indicators in assessing the health status of animals, providing insights into their nutritional, physiological, and pathological conditions (Abd El-Hack and Alagawany, 2015). The hematological analysis's output showed that the addition of thyme, rosemary, and oregano powder at equal levels in broiler diets increased significant impacts on Hb, TEC, and PCV. Rosemary mainly raises Hb levels in broiler chickens by lowering oxidative stress, improving food absorption (particularly iron), promoting blood cell synthesis. Because of these benefits, it makes it an effective natural additive for raising haematological markers and general productivity in poultry diets. The presence of Hb is the protein in red blood cells that binds and carries oxygen from the lungs to tissues and organs and, higher levels of TEC increase the number of red blood cells accessible for oxygen transport. The increase in red blood cell production would naturally result in a higher PCV because the proportion of red blood cells in the blood is increased. Higher Hb levels and more red blood cells supply adequate oxygen to maintain metabolic processes. Growth and development depend on cells to efficiently create energy (ATP) through aerobic respiration, which is made possible by adequate oxygen. There was no notable impact on ESR level, neutrophil,

eosinophil, lymphocyte, and monocyte count, although a numerical increase was observed. The possible reasons may be the addition of thyme, rosemary, and oregano in broiler diet might not cause enough acute or chronic immune activation to significantly alter these values. The health and function of immune cells may be affected by the herbs, but not adequate to cause notable changes in specific neutrophils, lymphocytes, etc. Although herbs contain anti-inflammatory qualities, their effects might not be strong enough to significantly alter ESR or white blood cell counts.

Like our findings, previous reports of Jameel *et al.* (2014) and Elnaggar *et al.* (2016) found that addition of thyme and rosemary powder in feed increased the RBC counts, hemoglobin, and PCV significantly. Conversely, Hasan and Awad (2017) observed no significant differences in the RBC counts, Hb, and PCV due to dietary thyme powder supplementation. Regarding neutrophil, eosinophil, lymphocyte, and monocyte count, Elnaggar *et al.* (2016) and Hasan and Awad (2017) found significant differences due to dietary rosemary and thyme powder supplementation. Ali (2014) found a numerical increase due to thyme supplementation, which is consistent with our findings. Hematological parameters like PCV, Hb and TEC may show greater sensitivity because they are directly affected by oxidative stress and nutritional deficiencies. We hypothesize that thymol, carvacrol, and rosmarinic acids are present in these herbs can enhance iron metabolism and promote erythropoiesis (RBC production) through their antioxidant and anti-inflammatory effects. These antioxidant qualities protect them from damage, extending their lifespan and enhancing their functionality. Based on these findings, farmers can take a message from this experiment that supplementation of herbs can enhance PCV, Hb, and TEC production, which transports better oxygen and improves growth rates, FCR and resilience to stress. These herbs can also protect red blood cells from oxidative damage due to their antioxidant properties and enhance the health and longevity of red blood cells, and reduced risks of anemia.

Serum Biochemical Assays

The dietary groups exerted a substantial influence on serum TC and LDL levels, exhibiting a substantial decrease compared to control groups. The Thyme, rosemary, and oregano contains some bioactive components which are responsible for the observed reduction in TC and LDL levels in broilers. These herbs are rich in phenolic compounds (e.g., thymol, carvacrol, and rosmarinic acid) with strong antioxidant properties. The liver is a vital organ for the creation and processing of cholesterol, and antioxidants aid in lowering oxidative stress there. Enzymes involved in the synthesis of cholesterol, such as 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), can be inhibited by reduced oxidative stress. It has been demonstrated that thymol and carvacrol block HMG-CoA reductase, the enzyme responsible for the rate-limiting step in cholesterol synthesis (Lee *et al.*, 2003). The herbs' phenolic compounds may increase the expression of LDL receptors on liver cells, increasing LDL clearance from the bloodstream. When it is circulated more quickly, the levels of LDL cholesterol are lowered in the bloodstream. The findings regarding TC and LDL level align with the previous findings of Ghazalah and Ali (2008); Fallah and Mirzaei (2016) and Hasan and Awad (2017). Tayeb *et al.* (2019) reported lower TC levels in thyme (5 g/kg) and rosemary (5 g/kg) supplemented groups. Similarly, Elnaggar *et al.* (2016) also found reduced TC and LDL contents in the rosemary-supplemented group compared to the control. Contrarily, Toghyani *et al.* (2010) found no significant changes in TC and LDL cholesterol due to thyme supplementation. Attia (2018) found same cholesterol levels in the 0.5% rosemary-supplemented group compared to the control. Concerning LDL levels, Ali (2014) observed no significant differences in LDL in thyme-supplemented groups compared to control.

Conversely, the supplementation with dietary thyme, rosemary, and oregano did not significantly alter the TG and HDL levels. Like our findings, Toghyani *et al.* (2010); Fallah and Mirzaei (2016); Hasan and Awad (2017) found no significant differences in TG levels in thyme-supplemented groups compared to the control. On the contrary, Attia (2018) and Elnaggar *et al.* (2016) found lower TG levels in the 0.5% thyme and rosemary supplemented groups. Regarding HDL levels, similar findings were reported previously by Ghazalah and Ali (2008) and Toghyani *et al.* (2010). On the contrary, Elnaggar *et al.* (2016) and Fallah and Mirzaei (2016) reported lower HDL levels, while Ali (2014) and Hasan and Awad (2017) observed higher HDL levels. Under typical dietary circumstances, the active ingredients in thyme, rosemary, and oregano (such as thymol, carvacrol, and rosmarinic acid) might not have a major impact on the pathways controlling triglyceride synthesis, utilisation and storage that's why TG levels remain stable. A balanced diet may already optimise the baseline levels of TG and HDL in healthy broilers that results in insignificant outcome. Another reason may be the presence of bioactive substances included in oregano, thyme, and rosemary that might not have much of an impact on the gut's ability to absorb fat, which is a major factor in determining the levels of triglycerides in the blood. TG levels are unaltered unless there is a substantial change in the digestion or absorption of fat. The findings on the effects of thyme, rosemary, and oregano supplementation lowered TC and LDL levels in broiler meat, which may have a healthier fat profile, that help to decrease the risk of cardiovascular disease. Furthermore, herbs are natural additives that provide health bene-

fits without relying on synthetic growth promoters or antibiotics and provide the medicinal properties. Not only that, but herbs are also being used as feed additives in poultry diets to support sustainable poultry farming by reducing reliance on chemical additives.

The liver is one of the most important organs in the body, as it performs various metabolic functions and detoxifies harmful substances. Higher amounts of ALT and AST enzymes are present in liver cells, which serve as bad indicators of liver health and function. Maximum levels of these enzymes in the serum may reflect liver damage or inflammation caused by various factors, such as infections, toxins, or stress (Nameghi *et al*., 2022). The findings of the current study indicated that thyme, rosemary, and oregano powder had no side effects on liver functions. Herbs have antioxidant properties, which play a protective role in preventing liver damage and, consequently, reducing ALT and AST levels in the serum. The bioactive components found in thyme and oregano (such as thymol and carvacrol) and rosemary (such as rosmarinic acid, rosmanol, carnosol, and caffeic acid), which prevent tissue damage (Hashemipour *et al*., 2013; Loetscher *et al*., 2013), play a crucial role as exogenous antioxidants. In line with the current study, Elnaggar *et al*. (2016) also reported reduced ALT and AST levels were found in the 0.5% rosemary powder-supplemented group. Tayeb *et al*. (2019), did not get any noteworthy variations in 5 g/kg thyme and rosemary supplemented groups, but a decreasing trend was observed. These consistent observations across studies suggest that these herbs may possess hepatoprotective properties, potentially capable of inducing endogenous interferon (Abd El-Latif *et al*., 2013).

Cost-Benefit Analysis

The results of this investigation made it abundantly evident that due to the absence of significant differences in BW and FI of the experimental broilers, no significant differences were found among the treatment groups in terms of feed cost, total cost, profit, and BCR. The non-significant difference in cost-benefit may be attributed due to the non-significant difference in growth performance among the treatment groups. If the data had a significant change in growth and a huge difference in herb's price, that could lead to a significant impact on cost-benefit analysis. Like our results, Hasan and Awad (2017) found that although the supplemented groups' growth performance was improved, the addition of thyme to the broiler diet had no discernible effect on net revenue or economic efficiency. Scaling up natural, AGP-free broiler meat production represents some opportunities and challenges like economic viability, production efficiency, disease management, consumer demand and market access and environmental and sustainability considerations. AGP-free broiler meat has reshaped profitability trends in the poultry industry, which results in rising consumer demand for safe broiler meat. Manufacturers can charge more for poultry meat that is devoid of AGP and is labelled as "natural," "organic," or "clean label." The possible cost increases from alternate management techniques, including supplementing with herbs, offering AGP free meat lowers reputational risks and increases consumer trust, which promotes profitability and long-term brand loyalty. AGP-free broiler production has significant practical implications for producers. Firstly, AGP-free broiler meat commands higher prices in health conscious and organic market. Second, herbs improve immunity and lower the incidence of disease, which eventually lowers veterinary costs and death rates. In the case of market competitiveness, natural or AGP-free meat meets the expectations of strengthening the market position. Further research may be directed with the optimal inclusion levels of herbs, synergistic effects of herb combinations, quality of herb and economic modeling is essential to make herb supplementation more cost-efficient in the poultry production.

CONCLUSIONS AND RECOMMENDATIONS

The findings of the present study showed that inclusion of rosemary powder into broiler diets yields a positive impact on growth performance of broiler chickens, WHC of meat and hemoglobin levels. Supplementation of thyme in the broiler diet significantly reduced TC and TG levels. Oregano powder has a vital impact on yellowness of the meat, and liver enzymes for maintaining better health conditions. Furthermore, herb-added groups enhanced the profit margin in the production of commercial broilers. In this current study, it is therefore recommended that 5g/kg of thyme, rosemary and oregano powder may be supplemented in broiler diets to improve lipid profiles, growth performance and liver enzymes, respectively. By adding thyme, rosemary, and oregano to broiler diets, farmers can increase growth performance, lower expenses, enhance meat quality, and take advantage of consumer preferences for natural and antibiotic-free poultry. These modifications not only boost profitability but also contribute to a sustainable and competitive farming business. Future research should be directed on synergistic interaction between active compounds and may be applied 10-15 g of herb powder per kg of feed to study the long-term effects.

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NOVELTY STATEMENTS

In the context of Bangladesh, where the poultry industry is a significant contributor to the economy, the use of natural feed additives like thyme, rosemary, and oregano could offer a sustainable alternative to synthetic growth promoters. These herbs are not only effective but also align with consumer preferences for natural products.

AUTHOR'S CONTRIBUTIONS

The experiment was designed by Shubash Chandra Das, Anguara Khatun and Bibek Chandra Roy. Nusrat Jahan Nishat recorded all kinds of data. The manuscript was drafted by Anguara Khatun and Ankon Lahiry. For manuscript revision, all authors contributed equally and approved the submitted version.

Conflict of Interest

No competing interests are disclosed by the authors.

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