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Fish oil, Azadirachta indica and Curcuma longa improve feed efficiency and meat quality of the broiler chicken

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Fish oil, *Azadirachta indica* and *Curcuma longa* improve feed efficiency and meat quality of the broiler chicken

Running title: Effects of *Azadirachta indica* and *Curcuma longa* on broiler chicken

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Abstract

Total 288 Ross-308 male broiler chicks were randomly distributed in a complete block design at 2×3 (Two different phytochemicals, i.e., *Azadirachta indica*, and *Curcuma longa* at three different levels, i.e., 0, 0.063, and 0.125% of the basal diet) factorial arrangement. Final live weight (FLW), average daily feed intake (ADFI), average daily gain (ADG), feed efficiency (FE), carcass characteristics, cardio-pulmonary morphometry, haemato-biochemical indices, gut morphology, ileal nutrient digestibility, tibia morphometry, meat quality and fatty acid profile were measured. Results indicated that, supplementation of *Azadirachta indica* leaf meal (AILM) decreased the FLW, ADFI, ADG, gizzard weight, right ventricular diameter and increased the FE, tibia calcium content, left ventricular weight and the ratio of right and left ventricle. The AILM substantially increased the malonaldehyde concentration in the Pectoralis major muscle of the broiler chicken at 7th and day 14th days. The *Curcuma longa* powder (CLP) decreased the FLW, ADFI and ADG without affecting the FE and increased the weight of right ventricle, left ventricle and tibia length. The AILM and CLP interacted to decrease the ADFI, total saturated fatty acid content and increase the FE, ω-6, ω-9, total unsaturated fatty acids, total poly-unsaturated fatty acids and the ratio of total unsaturated: saturated fatty acid in the breast muscle of the broiler chicken. It was concluded that *Azadirachta indica* and *Curcuma longa* in combination with fish oil improved the FE and meat quality of the broiler chicken at the expense of ADFI and ADG.

Keywords: Broiler, cardio-morphometry, fatty acid profile, performance

Introduction

Fish oil is a vital source of dietary n-3 polyunsaturated fatty acids, in particular, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which have potential health benefits (Weitz et al., 2010; Swanson et al., 2012; Goel et al., 2018). Several attempts have been made to increase the levels of these fatty acids in the widely consumed animal products specially poultry meat (Farhoomand and Checaniazer, 2009; Saleh et al., 2010; Abd El-Wahab, 2017; Lokapirnasari et al., 2017). Fish oil containing high amount of EPA and DHA has the capacity to enrich broiler meat. The previous studies indicated that the inclusion of fish oil in the diets exhibited no adverse effects on the productive performance of the birds in terms of feed intake, weight gain, feed efficiency and mortality as compared with vegetable oils (Saleh et al., 2010; Abd El-Wahab, 2017).

The *Azadirachta indica* (Neem) is a popular medicinal herb for about 5000 years. It is an important source of more than 130 different biologically active compounds having effective anticancer, antiviral, antibacterial, antifungal, antihypertensive, antiulcer, antidiabetic, antiasthma, antioxidant, antinephrotoxic, antimalarial, immunomodulatory, neuroprotective, hepatoprotective and wound healing activity (Biswas et al., 2002a; b; Herrera-Calderon et al., 2019). The *Azadirachta indica* plays therapeutic role because of a number of active constituents, i.e., nimbin, nimbidin, nimbanene, nimbidol, nimbolinin, nimbinate, gedunin, salannin, quercetin and ascorbic acid (Hashmat et al., 2012; Kharwar et al., 2020). Recently the quercetin, ß-sitosterol, and polyphenolic flavonoids have been purified in the laboratory from fresh leaves which have vital health benefits.

The *Curcuma longa* (Turmeric) is a spice that is commonly used in Asian foods for flavor and color. It contains a yellow color bioactive compound 'curcumin' which has proven antiinflammatory, antibacterial, antimicrobial, and antioxidant properties that block the action of inflammatory molecules in the body. The curcumin has further been proven to be an active scavenger of free radical which causes damage of body cells, tissues and organs. Thus, 'curcumin' damages free radical, reduces inflammations and prevents cardiovascular disorders. In addition to antioxidant activity, turmeric has been proven to lower cholesterol, triglycerides, and blood pressure (Qin et al., 2017; Hadi et al., 2019).

Overall, the *Azadirachta indica* and *Curcuma longa* are natural medicinal herbs that can be used as feed additives for improving performance of farm animals. Compared with synthetic antibiotics and inorganic chemicals, these plant products have been proven to be natural, less toxic, and residue free feed additives in food animal production. Most of the previous studies looking at the efficacy of these herbal products have been small trials with inconsistent results. Studies relating to the factorial combinations of fish oil, *Azadirachta indica* and *Curcuma longa* for commercial broiler production and its subsequent effect on meat quality of broiler are scant. We therefore aimed to explore the effects of fish oil, *Azadirachta indica*, and *Curcuma longa* on performance, carcass characteristics, cardio-pulmonary morphometry, haemato-biochemical indices, ileal nutrient digestibility, gut morphology, tibia morphometry, oxidative stability and fatty acid profile of meat in commercial broiler chicken.

Materials and methods

Study design, birds, and housing

Total 288 Ross-308 day old male broiler chicks were randomly distributed in a complete block design at 2×3 (Two different phytochemicals, i.e., *Azadirachta indica*, and *Curcuma longa* at three different levels, i.e., 0, 0.063, and 0.125% of the basal diet) factorial arrangement. Each treatment had four replicates containing eight birds per pen (Table 1). The trial was conducted during April-May 2021 in the experimental poultry shed of Chattogram Veterinary and Animal Sciences University (CVASU), Khulshi, Chattogram-4225, Bangladesh. Birds were purchased from Nahar Agro Limited, Chattogram, Bangladesh. All chicks were examined for abnormalities and uniform size. The shed was of brick cemented with corrugated metal wiring at bottom. Floor space for each bird was 1 square feet. The shed was thoroughly cleaned and washed by using tap water with caustic soda. For disinfection, phenyl solution (1% v/v) was sprayed on the floor, corners, ceiling, brooding boxes, and rearing cages. After cleaning and disinfection, the house was left empty 24 h for proper drying. After drying, all doors and windows were closed and the entire shed was fumigated overnight with single strength gas fumigant (40 ml formalin + 20 g $KMnO_4$ for 100 cubic feet area). On the next day, lime was spread on the floor and around the shed. Footbath containing potassium permanganate (1% w/v) was kept at the entrance of the poultry shed and changed daily. Feeders and drinkers were cleaned and washed with Timsen® solution (0.3% v/v) daily. The chicks were brooded at a recommended temperature of 95 °F, 90 °F, 85 °F and 80 °F for the 1st, 2nd, 3rd and 4th weeks, respectively with the help of incandescent bulbs. All birds were vaccinated against Newcastle disease at 5th and 12th days and Infectious Bursal Disease at 17th and 22nd days. The entire experimental protocol was approved by the Animal Welfare Law in Bangladesh (Memo No. CVASU/Dir(R&E)EC/2021/244(4)).

Experimental diet

The *Azadirachta indica* fresh leaf was collected, air dried to prepare *Azadirachta indica* leaf meal (AILM). The *Curcuma longa* was purchased, dried and ground to *Curcuma longa* powder (CLP). Feed ingredients were purchased from local market. Dry mash feed was provided to the birds throughout the whole experimental period. Nine different types of rations were formulated as per requirement of the Ross-308 (Table 1-2). Each ration was of two different types, i.e., starter (1-14 d) and finisher (15-35 d). All rations were *iso*-caloric and *iso*-nitrogenous. Feed was prepared manually and supplied *ad-libitum* to the birds on round small feeder for 1-7 day. After 7th day, small round feeders and waterers were replaced by linear feeders and bell drinkers.

Performance parameter

Birds were inspected daily for mortality and recorded as it occurred. The dead birds were subjected to post-mortem examination, removed and weighed for subsequent correction of weight gain. Body weight and average daily feed intake (ADFI) were recorded at 14th and 35th day for calculation of average daily gain (ADG), and feed efficiency (FE). Weight gain was calculated by deducting initial body weight from the final body weight of the birds. Feed intake was calculated by deducting leftover from the total feeds supplied to the birds. FE was calculated by dividing feed intake with weight gain.

Chemical analysis

All proximate components were analyzed as per standard procedure (AOAC, 2019). Moisture was estimated by Hot air oven (SLN-115, Pol-Eko-Aparatus SP. J, Poland). Crude protein was estimated by micro Kjeldhal apparatus (Kjeldhal digestion unit SBS800, Kjeldhal distillation unit D1000, FoodAlyt, Germany). Crude fibre was estimated by using Ankom Fiber Analyzer (Fiberbag System-6, Gerhardt, Germany). Ether extract was estimated by using Soxtec (RS-

232, SER-148, VelpScientifica,155 Keyland Court, Bohemia, NY 11716 - US). Ash was estimated by muffle furnace (HYSC, Non-Yong Scientific Equipment Company Ltd., 874-1 Wolgye 4-dong, Nowon-gu, Seoul, Korea). Gross energy of diets, *Azadirachta indica*, *Curcuma longa* and digesta was determined by using the bomb calorimeter (Parr 6200 Calorimeter, Parr Instruments Co., USA).

Hemato-biochemical tests

All biochemical tests were conducted by a hemato-biochemical analyzer (HumaLyzer 3000, Human, Germany). Manufacturer's recommended standard test kits were used for testing serum glucose (Method: GOD-PAP, Ref. 10260, Liquicolor, Human, Germany), SGOT (Method: ALAT IFCC, Ref. 12021, Human, Germany), SGPT (Method: ALAT IFCC, Ref. 12022, Human, Germany), urea (Ref. 1156010, Linear chemicals, Barcelona, Spain), uric acid (Ref. 10690, Human, Germany), creatinine (Ref. CR 510, Randox, UK), albumin (Ref. 10560, Human, Germany), total protein (Ref. 10570, Human, Germany), tri-glyceride, TG (TR 210, Randox, Germany), low density lipoprotein, LDL (Ref. BXC 0432A, Biorex Diagnostic Ltd., UK), high density lipoprotein, HDL (Ref. CH 203, Randox, Germany), total cholesterol (Ref. CH 200, Randox, Germany), calcium (Ref. CA 590, Randox, Germany), phosphorus (Ref. PHO-012, Randox, Germany) and T_4 (Ref., Bioscience medical, Madrid, Spain).

Digestibility trial

At the age of day 28, one replicate containing eight birds from each treatment was selected for digestibility trial. The selected birds were provided feed and water *ad libitum*. For consecutive three days pre-slaughter (28-30 days), titanium oxide (TiO_2) was fed at 5 g/kg as an indicator for measurement of nutrient digestibility (Short et al., 1996). Three birds from each replicate were slaughtered humanely at the end of 30th day by cervical dislocation of jugular vein and carotid artery 3 hour after feeding. The pooled ileal digesta were collected from the Meckel's diverticulum to the ileal-cecal-colon junction avoiding reflux of the large intestinal digesta, poured in a small plastic bag and stored immediately at -20ºC. The digesta was freeze-dried, ground through 0.25 mm sieve, and mixed thoroughly for analysis. Dry matter, organic matter, crude protein, crude fibre, ether extract, ash, calcium, and phosphorus were determined in the feed and ileal digesta according to standard method (AOAC, 2019). Titanium oxide of the experimental diet and ileal samples were determined by the UV-VIS Spectrophotometer (UV

2600, Shimadzu, Japan). Apparent ileal nutrient digestibility (AID) was determined according to standard procedure (Maynard, 2018) by using the following formula: AID% = 100 – [(feed indicator %/ileal indicator %)× (ileal nutrient %/feed nutrient %) × 100]. All samples were analyzed in the Postgraduate laboratory of the department of Animal Science and Nutrition, CVASU.

Carcass characteristics

On day 30, two birds from each replicate cage weighing average pen weight were selected to record their carcass characteristics. Blood samples were collected while slaughtered for assessing plasma and serum metabolites. After bleeding, the birds were defeathered, eviscerated, and head and feet were discarded to determine dressed carcass weight. The whole carcass was processed accordingly (Jones, 1984) to assess carcass characteristics (dressed yield, breast weight, thigh weight, drumstick weight, shank weight, and other). Relative weight of visceral organs, i.e., gizzard, proventriculus, and abdominal fat (from the surrounding proventriculus and the gizzard down to the cloaca) was also recorded.

Cardio-morphometry

The heart was isolated from the carcass immediate after slaughter. The data of heart weight, right ventricular weight, left ventricular weight, right ventricular diameter, left ventricular diameter, right ventricular thickness and left ventricular thickness were measured thereafter by using slide caliper (Wiika Vernier Caliper, 150mm, WA-VC1150) and digital screw gauge (Mitutoyo, Quickmini, Mitutoyo corporation, Japan).

Tibia morphometry

Both right and left legs were boiled for 5 minutes to loosen muscle and connective tissue. Using scissors, forceps, scalpel, and cheesecloth, meat, connective tissue, and the fibula bone were completely removed, leaving the complete tibio-tarsus with external cnemial crest, lateral condyle (malleolus) of tibia and the intact ossified tibial cartilage (Lucas and Stettenheim, 1972). As the bones were cleaned, they were put into a container of ethanol and soaked for 72 h to remove water and polar lipids. Bones were then extracted in an hydrousether for 6 h in the Solvent extractor (SX-6MP, Raypa, France). After the second extraction, bones were dried at room temperature for 24 h. Tibia morphometry, i.e., length, diameter, weight was recorded. The tibia was then ground and ashed in a muffle furnace (HYSC, Non Yong Scientific Equipment Co. Ltd., 874-1 Wolgye 4-dong, Nowon-gu, Seoul, Korea) overnight at 600º C, and weighed again after ashing to calculate tibia ash.

Gut morphology

At day-30, two birds from each of 36 replicates were randomly selected for slaughter. The gastrointestinal tract was removed and separated into the three intestinal segments, i.e., duodenum, jejunum and ileum. The length, weight and diameter of duodenum were taken distally from gizzard to the end of pancreatic loop, for jejunum from pancreatic loop to the Meckel's diverticulum and for ileum the Meckel's diverticulum to the ileo-caecal junction. All measurements were triplicate and averaged later on.

Fatty acid profile

Lipids were extracted from the breast muscles (pectoralis major and pectoralis minor) of the slaughtered birds using chloroform/methanol (1:1 v/v) by a modified method (Folch et al., 1957). Fatty acid methyl esters were prepared for gas chromatography determination using KOH/methanol (Luo et al. 2009). The Gas Chromatograph (Nexis, GC-2030, Shimadzu, Japan) equipped with a robotic auto sampler (AOC 6000), a hydrogen flame ionization detector, and quartz capillary chromatographic column HP-5MS (30 m × 0.25 mm ×0.25 µm) was used in this experiment. The injector was set at 150°C. Nitrogen was used as carrier gas with a flow rate of 1.0 ml/min, split ratio was 1:10. The column was programmed as follows: 60°C for 3 min, increase to 260°C (4°C/min), and held constant for 50 min. The fatty acids were identified by comparing the area of the peaks of the sample with that of known standards (SigmaAldrich, St. Louis, USA).

Statistical analysis

Data related to feed intake, weight gain, FCR, nutrient digestibility, carcass characteristics, cardio-morphometry, gut-morphometry and hemato-biochemical indices were taken from every pen. The individual pen was considered as the independent experimental unit. Data were tested for outliers and multicollinearity by inter quartile range test and variance inflation factors. Normality of response variable was checked by using normal probability plot and equality of variances in the response variable was checked by Shapiro Wilk test. Data were analyzed for generalized linear model by using Stata 14.1 SE (Stata Corp LP, College Station, Texas, USA). Kaiser-Meyer-Olkin measures of sampling adequacy and Bartlett's test of sphericity were applied to test the suitability of the dataset for the principal component analysis (PCA) in the SAS platform (SAS JMP Pro 16.0). Two principal components were estimated following orthogonal 'varimax' rotation (Kaiser off) based on maximum 'eigen' values obtained from the 'scree plot'. Discriminant factors were classified as canonical 1 and 2 plotted on 'x' and 'y' axes and tested by using Wilk's Lambda, Pillai's Trace, Hotelling-Lawley and Roy's Max Root test. Normal mixture clusters were analyzed in the scatterplot matrix assuming that a mixture of overlapping multivariate could have similar normal distributions. Finally, response surface models with central composite design (CCD) were applied in factorial arrangements to optimize the desirable zone of responses. While means were deemed significant for GLM, the Duncan's New Multiple Range Test (Ha et al., 1990) was applied to partition them. Statistical significance was accepted at $p<0.05$ for Fisher's F-tests. The following statistical model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where,

μ = The intercept of the regression model;

α_i = The fixed effect of the 'i^{th}' level of the factor 'α' (*Azadirachta indica*) on the value observed in Y_{ijk} (i=0, 0.063, 0.125% of diet);

β_j = The fixed effect of the 'j^{th}' level of the factor 'β' (*Curcuma longa*) on the value observed in Y_{ijk} (j=0, 0.063, 0.125% of diet);

$(\alpha\beta)_{ij}$ = The interaction effect of the of the 'i^{th}' level of the factor 'α' and the 'j^{th}' level of the factor 'β';

Y_{ijk} = The observed effects of the variable under study at the 'i^{th}' level of the factor 'α' and the 'j^{th}' level of the factor 'β'for the 'k^{th}' repetition of measurements;

ε_{ijk} = The random sampling error due to 'i^{th}' level of the factor 'α' and 'j^{th}' level of the factor 'β' at the 'k^{th}' repetition of measurements.

Results

Performance

Supplementation of *Azadirachta indica* leaf meal (AILM) decreased ($p<0.001$) the FLW, ADFI and ADG at 1-14 d, 15-35 d and 1-35 d. The FE improved at 1-14 d ($p<0.001$), 15-35 d ($p<0.01$) and 1-35 d ($p<0.001$). The *Curcuma longa* powder (CLP) decreased the FLW ($p<0.05$), ADFI ($p<0.001$) at 15-35 d, 1-35 d and ADG ($p<0.05$) at 1-14 d and 1-35 d without affecting the FE. The AILM and CLP interacted to decrease the ADFI ($p<0.05$) and improve the FE ($p<0.05$) at 1-35 d (Table 3, Figure 1-3). Principal component analysis identified feed FI, ADG and FE as main eigen vectors controlling variability of the performance of the broiler chicken (Figure 4). Increased dietary concentration of AILM and CLP optimized FE (Figure 5).

Carcass characteristics

The AILM increased ($p<0.05$) only the neck weight. All other carcass components remained unchanged due to supplementation of AILM and CLP (Table 4; Figure 6).

Cardio-pulmonary morphometry

The AILM decreased ($p<0.05$) the right and left ventricular diameter and increased ($p<0.05$) the ratio of right to total ventricle. The CLP increased ($p<0.05$) the weight of the right, left and total ventricles. There were no interaction effects of AILM and CLP (Table 5). Discriminant analysis identified distinctive role of AILM and CLP on cardiopulmonary morphometry (Figure 7) of the broiler chicken which was optimized by the lower dietary concentration of AILM and CLP (Figure 8).

Haemato-biochemical indices

The main or interaction effects of AILM and CLP appeared nil ($p<0.05$) on hemato-biochemical indices (Table 6).

Gut morphology

The AILM decreased ($p<0.05$) 10.9% of the gizzard weight leaving all other gut morphology unchanged ($p>0.05$) (Table 7).

Ileal nutrient digestibility

The AILM increased 9.6% of the digestibility of CP. The CLP decreased 12.8% of the digestibility CF. The AILM and CLP interacted to increase 16.8% of the digestibility of CP and 7.2% of the digestibility of EE at the expense of 10.9% reduction of the digestibility of CF (Table 8).

Tibia morphometry

The AILM increased ($p<0.01$) 35.9% tibia calcium content at the expense of 6.8% total ash. The CLP increased ($p<0.001$) the tibia length (6.4%), diameter (14.7%) and weight (32.5%). The AILM and CLP interacted to decrease ($p<0.001$) 5.5% total ash (Table 9).

Meat quality

The AILM decreased 30.6% NFE and 28.0% EE but increased 93.2% MDA at day 0 ($p<0.01$) and 137.5% MDA at day 7 ($p<0.001$) in the Pectoralis major muscle of the broiler chicken. The CLP decreased 6.8% total ash (Table 10).

Fatty acid profile

Supplementation of AILM substantially increased 52.4% UFA, 58.2% MUFA, 38.7% ∑PUFA, 41.7% ∑ω-6 fatty acids, 69.7% ∑ω-9 fatty acids and decreased 24.3% ∑SFA. Similarly, supplementation of CLP increased 8.1% ∑UFA, 21.6% ∑PUFA, 22.4% ∑ω-6 fatty acids and decreased 4.3% ∑SFA. AILM and CLP interacted to increase 35.3% ∑UFA and to decrease 21.2% ∑SFA (Table 11).

Discussion

Performance

In our study, supplementation of *Azadirachta indica* leaf meal (AILM) and fish oil to the broiler diet substantially reduced average daily feed intake (ADFI) and average daily gain (ADG) although feed efficiency (FE) improved significantly. Consistent with our result, Landy et al. (2011) and Shivappa Nayaka et al. (2013) reported retarded growth rate while fed 7-12 g/kg neem leaf to the broiler chicken. Accordingly, Ubua et al. (2019) and Bonsu et al. (2012) observed impaired growth due to incorporation of AILM in broiler diet although in few cases no marked differences were noticed in the FLW and ADG (Wankar et al., 2009; Shaahu et al., 2020).

Contrasting with our findings, improved FLW and ADG were reported in a series of studies (Onyimonyi et al., 2009; Ansari et al., 2012; Paul et al., 2020). Obun et al. (2013) reported improved FE in broiler chicken feeding AILM which is aligned with our study. The reduction in ADFI, thereby, ADG could be attributed to the presence of bio-active compound 'azadirachtin' which is responsible for the bitter and unpleasant taste of neem leaf. Accordingly, the high fibre content of neem leaf adversely affects the feed intake and digestibility of nutrients in broiler chicken (Esmail, 2012). Additionally, the presence of anti-nutritional factor terpenes and limonoids in neem leaf may have adverse effect on ADFI and ADG (Kabeh and Jalingo, 2007; Ogbuewu et al., 2011). On the other hand, the improved FE can be explained by the presence of a series of active ingredients in neem leaf (Hashmat et al., 2012; Kharwar et al., 2020).

Our study further elucidated that *Curcuma longa* powder (CLP) decreased the live weight, ADFI and ADG while FE was unaffected. Similar to our study, Wang et al. (2015), Nouzarian et al. (2011) and Mehala & Moorthy (2008) observed no differences in the body weight of the broiler chicken when the diet was supplemented with turmeric powder. Closely consistent results were reported somewhere else indicating no increase in the FLW and ADG with turmeric supplemented diet (Arslan et al., 2017; Johannah et al., 2018).

The 'curcumin' an active ingredient isolated from turmeric is a natural polyphenol (Anderson et al., 2000) proven for increased body weight in broiler chicken (Rajput et al., 2013). Like 'curcumin', many other factors may influence body weight and ADG, i.e., nutrition, management and environmental factor. A significant decrease in ADFI could be the reason of lower body weight and ADG in broiler. Previous studies reported that turmeric powder at 0.5% of the diet reduced ADFI in the broiler chicken (Mehala and Moorthy, 2008; Daramola, 2020). The reduction in ADFI may be because of unpleasant turmeric smell. Moreover, feeding mash feed tends to reduce ADFI (Aguzey et al., 2018). Similar to our study, FE remained unchanged in a previous study (Shivappa Nayaka et al., 2013). Most probably, reduced ADFI attributed to the reduced ADG resulting unchanged FE.

The interaction effect of AILM and CLP decreased the ADFI and improved the FE which is consistent with Shivappa Nayaka et al. (2013) where ADG concomitantly decreased with neem, turmeric and vitamin E supplementation. The ADFI was affected by the taste and smell of the herbs we used in our study. However, the active ingredients present in these plants might have improved feed efficiency by influencing intestinal microflora and thereby increased nutrient absorption (Rajput et al., 2013; Ubua et al., 2018).

Carcass characteristics

The AILM increased only the neck weight in our study leaving all other carcass components unchanged. These results closely resemble the findings of Egbeyale et al. (2020) who reported that the dressing percentage and all other organ weight except back weight were unaffected due to incorporation of AILM in the broiler diet. Khatun et al. (2013) reported no difference in the carcass characteristics when AILM and tulshi (*Ocimum tenuiflorum*) were added to the broiler diet. However, neem leaf extract improved dressing percentage of the treated group compared with negative control (Paul et al., 2020). The study further reported increased heart, spleen and pancreas weight. The differences might be because of different inclusion level of neem extract in diet since higher doses may have higher toxic effect of terpenes and limonoids causing hepatomegaly.

Previous study (Mehala and Moorthy, 2008) further illustrated that turmeric, aloe vera or their combinations had no effect on carcass characteristics of broiler chicken which strongly supports our study. Contrastingly, all the carcass traits and organ weights including the neck weight were significantly affected due to supplementation of turmeric in broiler ration in a series of studies (Hussein, 2013). Additionally, turmeric enhanced dressing percentage, abdominal fat and organ weight (Nouzarian et al., 2011; Wang et al., 2015; Attia et al., 2017).

The discrepancies could be explained by the differences in the inclusion level and the 'curcumin' content of turmeric in the diet (Asghari et al., 2009). However, since no organ, especially liver and pancreas were enlarged, it was indicative that the anti-nutritional factors were minimum in the test ingredients.

Cardio-pulmonary morphometry

In our study, the AILM decreased the right ventricular diameter increasing the left ventricular weight and the ratio of right and left ventricle. The CLP increased the weight of right ventricle decreasing the weight of left ventricle, total ventricle and left ventricular diameter. Literature related to direct effects of AILM and CLP supplementation on cardio-pulmonary morphometry of broiler chicken are scant. Hence, relying upon our result, it could indirectly be inferred that supplementation of AILM was beneficial for the cardio-pulmonary vasculature of the broiler chicken.

Haemato-biochemical indices

In our study, either main or interaction effect of AILM and CLP on hemato-biochemical indices appeared nil. Similar result was reported in a previous study where hematological parameters remained unchanged due to supplementation of AILM in broiler diet (Paul et al., 2020). In contrast to our study, serum glucose, cholesterol, protein, albumin, urea, creatinine, ALT and AST were significantly affected by the incorporation of AILM (Obikaonu et al., 2012). Closely similar result was reported in another study by Nnenna & Okey (2013) who mentioned that although total protein, albumin, urea and creatinine remained unchanged, glucose and cholesterol levels differed due to supplementation of neem extract. Supplementation of AILM in rabbit diet had no effect on protein, albumin, creatinine, ALT and AST, although, urea, cholesterol and glucose levels were influenced (Ogbuewu et al., 2010). It was argued that the AILM indirectly inhibited HMG-COA reductase which induced hypoglycemic activity that altered the physical state of the animals. Mehala & Moorthy (2008) observed no changes in the haemato-biochemical parameters while supplemented turmeric in broiler diet. In contrast to our study, Attia et al. (2017) reported that serum biochemical parameters were altered in the treatment groups compared with control. The primary active ingredient of turmeric is 'curcumin' which is reported to exert hypocholesterolemic and hypolipidemic effect (Ahmad et al., 2020). However, in our study the effects were not noticed vividly perhaps because of dose differences although serum biochemistry remained within standard range indicating normal health.

Gut morphology

The AILM decreased only the gizzard weight. The CLP had no main or interaction effect on gut morphology. Similar result was reported by Landy et al. (2011) who observed no effect of neem leaf extract on gut morphology. Accordingly, Egbeyale et al. (2020) observed no difference in the weight of the small intestine, large intestine and caecum. Hernandez et al. (2004) found no difference in gut weight among control, antibiotic and plant extract treated groups compared with control. Previous result suggested that gizzard weight increased with the supplementation of mash feed (Aguzey et al., 2018) although mash feed with large particle size had negative effect on gizzard weight for the young birds (Charbeneau and Roberson, 2004; Parsons et al., 2006; Xu et al., 2015). The findings of our study further agree with Mustafa et al. (2021) who found that addition of turmeric in broiler diet had no effect on gizzard or intestine weight. Yesuf et al. (2017) reported that the weight of gastro-intestinal tract remained unchanged in turmeric treated groups which supports our study.

Ileal nutrient digestibility

Our study revealed that the digestibility of CP increased due to addition of AILM although CLP reduced the digestibility of CF. The interaction effect of AILM and CLP appeared positive for CP and EE but negative for CF, NFE, TA and phosphorus availability. Our results support the findings of Shaahu et al. (2020) who reported that the digestibility of CP was higher in aqueous neem leaf extract supplemented group compared to control. Previous study also revealed that *Azadirachta indica*, *Cichorium intyebus* and *Moringa oleifera* extract supplementation in broiler diet increased the digestibility of CP (Mahmood et al., 2015). It was speculated that, addition of AILM in broiler diet might have increased amino acid digestibility which in turn enhanced the digestibility of CP (Mahmood et al., 2015).

The apparent utilization of DM and CP appeared unaffected when the broiler chicks were supplied with direct ‘curcumin’ (Rajput et al., 2013). Again, use of turmeric nanocapsule improved nutrient digestibility of broiler (Yuwanta and Martien, 2014). This variations in the results may be because of the nanocapsule formulation which in turn increased the bioavailability and concentration of curcumin. It is proven that 'curcumin' has inhibitory effect on the movement of intestine when applied in higher doses (Purwar et al., 2012). The movement of intestine greatly influences the digestion of nutrients. Furthermore, the ant-nutritional factors present in turmeric like oxalate, phenol, alkaloid and glycoside may bind the nutrients and minerals making them bio-unavailable (Harbor, 2020).

The interaction effects of neem and turmeric appeared to improve the availability of calcium and phosphorus. The combination of plant extracts in broiler diet is proven to improve the digestibility of nutrients (Hafeez et al., 2020). The synergistic effect of AILM and CLIP in our study, thus, reflects similar phenomena despite mutual inhibition of anti-nutritional factors.

Tibia morphometry

The AILM increased the tibia calcium while CLP increased the tibia length, diameter and weight, although, the interaction effects of AILM and CLP appeared nil. A study in pig with herbal mix showed that neem mix had no effect on tibia weight, length and ash weight except decreased tibia thickness compared with control (Njoku et al., 2021). The result is partially in line with our study. Broiler chickens having fast growth are prone to leg problems. Higher calcium content of tibia in neem supplemented birds indicated better mineralization of bones compared to control. Neem leaf contains calcium and phosphorus along with other 13 different elements that may result in better mineralization of the bone (Sahito et al., 2003). Hosseini-Vashan et al. (2012) reported that the supplementation of CLP in heat stressed broiler increased the calcium content of tibia although total ash and phosphorus content remained unchanged. Our result showed increased length, diameter and weight of tibia without increasing the calcium and phosphorus content which implied that the bone became larger without increasing mineralization. Perhaps, the oxalate in turmeric was bound with calcium to form calcium oxalate which prevented it to be absorbed and utilized (Park and Nile, 2013; Harbor, 2020).

Meat quality

In a previous study, the pH of broiler meat fed either AILM or CLP and their combinations remained within normal range (Warris, 2000). Egbeyale et al. (2020) reported that inclusion of AILM in diet had no influence on pH of broiler meat. Daneshyar et al. (2011) further reported that the pH was not affected by the supplementation of turmeric in broiler chicken. These results are closely aligned with our findings. Contrastingly, Abubakar et al. (2021) reported that the pH of meat significantly differed among turmeric treated groups compared with control. The pH of meat is a crucial indication for the acceptability of meat to the consumer. The storage of glycogen in muscle is important to reach the ultimate pH of meat. Our results showed that AILM and CLP did not interfere the deposition of glycogen in muscle which was broken down to form lactic acid through anaerobic glycolysis (Warris, 2000).

The concentration of malonaldehyde (MDA) is commonly accepted as an indication of lipid peroxidation. The AILM substantially increased the malonaldehyde concentration in the Pectoralis major muscle of the broiler chicken at 7th and 14th days. Nakamura et al. (2021) reported that dietary supplementation of neem leaves reduced MDA concentration in broiler meat. Ouerfelli et al. (2019) reported that incorporation of neem leaves with beef patties decreased MDA concentration to reduce lipid peroxidation. The phenolic compound gallic acid and ferulic acid as well as flavonoids in neem have antioxidant properties which can decrease the MDA concentration in meat (Singh et al., 2005; Pandey et al., 2014). Daneshyar (2012) reported that dietary consumption of turmeric did not affect the MDA concentration in fresh meat but reduced MDA at 7th day. Turmeric contains curcuminoids, such as curcumin, demethoxycurcumin and bisdemethoxycurcumin which exert antioxidant activity (Cousins et al., 2007). In the present study, neem and turmeric leaf supplementation in broiler diet increased unsaturated fatty acids in meat. Despite the presence of antioxidant compounds in neem and turmeric which scavenge reactive oxygen species to inhibit lipid peroxidation, perhaps, the MDA concentrations were higher in AILM and CLP supplemented groups because of the increased amount of unsaturated fatty acids in meat which were prone to higher lipid peroxidation (Rael et al., 2004).

Fatty acid profile

The *Azadirachta indica* leaf meal and *Curcuma longa* powder synergistically interacted to increase the ω-6, ω-9, total unsaturated fatty acid (UFA), total poly-unsaturated fatty acid (PUFA) and the ratio of UFA:SFA by decreasing the SFA contents in the breast muscle of the broiler chicken. Similarly, inclusion of 1% neem seed oil increased the proportion of polyunsaturated fatty acids particularly omega 3, omega 6 and palmitic acid in broiler meat (Trigueros et al., 2015). Daneshyar et al. (2011) reported that turmeric supplementation significantly lowered the total SFA and trans-valeric acid concentration although other fatty acids remained unchanged. The concurrent action of AILM and CLP might have reduced SFA by increasing UFA in broiler meat. The active ingredient of turmeric ‘curcumin’ has hypolipidemic effect which in turn reduced the SFA concentration in meat. Dietary consumption of high amount of SFAs are associated with the risk of cardiovascular disease (Briggs et al., 2017). Thus, it could be inferred that the incorporation of AILM and CLP in broiler diet may have greater health benefits.

Conclusion

Supplementation of *Azadirachta indica* improved feed efficiency, tibia calcium content and the ratio of right to left ventricle. The *Azadirachta indica* and *Curcuma longa* interacted to decrease total saturated fatty acid contents and increased the ω-6, ω-9, total unsaturated fatty acids, total poly-unsaturated fatty acids and the ratio of total unsaturated: saturated fatty acids in the breast muscle of the broiler chicken. It was concluded that fish oil, *Azadirachta indica* and *Curcuma longa* improved feed efficiency and meat quality of the broiler chicken at the expense of feed intake weight gain.

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Conflicts of interest/Competing interests

None.

Availability of data and materials

All the data used in the manuscript exclusively belongs to the mentioned authors which could be shared under reasonable ground.

Code availability

Not applicable.

Author’s contribution

Dr. Emran Hossain-Conceptualization, fund acquisition, ration formulation, data curation, principal component and discriminant analysis, GLM and response surface modelling, result interpretation and draft finalization. **Dr. Kona Adhikary**-Fatty acid profile of meat, proximate analysis, haemato-biochemical indices, write up of initial discussion. **Dr. Nasima Akter-** Project administration, animal trial, immunization of the birds, data collection and preparation of the initial draft. **Dr. Priunka Bhowmik**-Tibia morphometry, ileal nutrient digestibility, gut morphology, carcass characteristics, MDA of meat. **Dr. Nahid Sultan-** Data collection, resources, critical review and preparation of the draft. **Dr. Shilpi Islam-** Data collection, resources, critical review and preparation of the draft. **Dr. Goutam Buddha Das-** Data collection, resources, review and preparation of the draft. All authors read and approved the final manuscript.

Ethics approval

The entire experimental protocol was approved by the animal welfare law in Bangladesh (Memo No. CVASU/Dir(R&E) EC/2021/244(5).

Consent for publication

We, all the human participants involved in this research, consent to publish all the materials mentioned in the manuscript.

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Table 1. Design of the experiment

Dietary treatments		No. of treatments	No. of replicates	No. of birds
Azadirachta indica (%)	*Curcuma longa (%)*			
0	0	1	4	4 × 8=32
0	0.063	1	4	4 × 8=32
0	0.125	1	4	4 × 8=32
0.063	0	1	4	4 × 8=32
0.063	0.063	1	4	4 × 8=32
0.063	0.125	1	4	4 × 8=32
0.125	0	1	4	4 × 8=32
0.125	0.063	1	4	4 × 8=32
0.125	0.125	1	4	4 × 8=32
Total	-	9	36	= 288

Table 2. Formulation of finisher (15-35 d) ration for the experimental broiler birds

Ingredient	Dietary combinations[1]								
	$A_0 \times C_0$	$A_0 \times C_{0.063}$	$A_0 \times C_{0.125}$	$A_{0.063} \times C_0$	$A_{0.063} \times C_{0.063}$	$A_{0.063} \times C_{0.125}$	$A_{0.125} \times C_0$	$A_{0.125} \times C_{0.063}$	$A_{0.125} \times C_{0.125}$
Maize	59.8	59.8	59.8	59.8	59.8	59.7	59.8	59.7	59.7
DDGS[2]	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Fish oil	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75
AILM	0.000	0.000	0.000	0.063	0.063	0.063	0.125	0.125	0.125
CLP	0.000	0.063	0.125	0.000	0.063	0.125	0.000	0.063	0.125
Soybean meal	31.5	31.5	31.5	31.5	31.5	31.5	31.5	31.5	31.5
Meat & bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lime stone	1.06	1.02	0.98	1.02	0.98	0.94	0.98	0.94	0.91
Dicalcium phosphate	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
L-Lysine	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
DL-Methionine	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Vitamin premix[3]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Feedzyme[4]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Common salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Acidifier	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	100	100	100	100	100	100	100	100	100
Calculated									
Metabolizable energy[5]	3100	3099	3098	3099	3098	3098	3098	3098	3097
Crude protein	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0
Crude fiber	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19
Ether extract	5.40	5.40	5.40	5.40	5.40	5.40	5.40	5.40	5.40
Calcium	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93
Phosphorus	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Available phosphorus	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
Sodium	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Potassium	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Magnesium	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Manganese (mg/kg)	65.6	65.6	65.6	65.6	65.6	65.6	65.6	65.6	65.6
Zinc (mg/kg)	73.6	73.6	73.6	73.6	73.6	73.6	73.6	73.6	73.6
Copper (mg/kg)	15.1	15.1	15.1	15.1	15.1	15.0	15.1	15.0	15.0
Iron (mg/kg)	147	147	147	147	147	147	147	147	147

Lysine	1.23	1.23	1.23	1.23	1.23	1.23	1.23	1.23	1.23
Leucine	1.81	1.81	1.81	1.81	1.81	1.81	1.81	1.81	1.81
Iso-leucine	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87
Valine	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Threonine	1.11	1.11	1.11	1.11	1.11	1.11	1.11	1.11	1.11
Methionine	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51
Tryptophan	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Phenylalanine	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02
Cystine + methionine	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70

[1] A_0 = *Azadirachta indica* 0%; $A_{0.063}$= *Azadirachta indica* 0.063%; $A_{0.125}$ = *Azadirachta indica* 0.125%; C_0 = *Curcuma longa* 0%; $C_{0.063}$= *Curcuma longa* 0.063%; C_{50} = *Curcuma longa* 0.125nn%;

[2] DDGS = Distiller's Dried Grain Soluble;

[3] Per 2500 g contained: Beta-Carotene (Vitamin A) 12000000 IU, Cholecalciferol (Vit-D_3) 2400000 IU, Alpha-Tocopherol (Vit-E) 23 g, Menadione (Vit-K_3) 2 g, Thiamine (Vit-B_1) 2.5 g, Riboflavin (Vit-B_2) 5 g, Pyridoxine (Vit-B_6) 4 g, Nicotinic acid 40 g, Calcium-D-Pantothenete 12.5 g, Cobalamin (Vit-B_{12}) 12 mg, Folic acid 800 mg, Biotin (Vit-B_7) 100 mg, Cobalt 400 mg, Copper 10 g, Iron 60 g, Iodine 400 mg, Manganese 60 g, Zinc 50 g, Selenium 150 mg, DL-Mehionine 100 g, L-Lysine 60 g, Calcium 679.6 g;

[4] Per 100 g contained: Cellulase 20000 IU, Xylanase 200000 IU, Protease 20 IU, Amylase 40000 IU, Phytase 20 IU, Pectinase 1400 IU, Invertase 400 IU, Hemicellulose 500 IU, Lipase 20 IU, α-Galactosidase 100 IU;

[5] Metabolizable energy (kcal/kg).

Table 3. Initial live weight (ILW, g), final live weight (FLW, g), average daily feed intake (ADFI, g/bird/d), average daily gain (ADG, g/bird/d), feed efficiency (FE, ADFI/ADG) of the broiler birds fed diet supplemented with fish oil, *Azadirachta indica* leaf meal (AILM) and *Curcuma longa* powder (CLP) in different proportions

Treatment factors[1]	ILW (g/bird)	FLW (g/bird)	Performance parameter								
			ADFI (g/bird)			ADG (g/bird)			FE		
			1-14 d	15-35 d	1-35 d	1-14 d	15-35 d	1-35 d	1-14 d	15-35 d	1-35 d
AILM											
0	46.4	2130.5	27.8	134.8	89.9	26.4	81.6	59.5	1.05	1.65	1.51
0.063	46.5	2006.5	26.5	122.7	82.2	25.5	76.4	56.0	1.04	1.61	1.47
0.125	46.1	1812.6	23.8	106.6	71.4	24.4	67.9	50.5	0.98	1.57	1.42
CLP											
0	46.3	2034.1	26.6	126.2	84.3	25.9	77.4	56.8	1.03	1.63	1.48
0.063	46.2	1986.9	26.0	121.5	81.3	25.4	75.5	55.5	1.02	1.61	1.46
0.125	46.4	1928.4	25.4	116.3	77.9	25.0	73.0	53.8	1.02	1.59	1.45
AILM×CLP											
AILM$_0$ × CLP$_0$	46.3	2155.4	27.9	138.3	92.1	26.8	82.6	60.3	1.04	1.68	1.53
AILM$_0$ × CLP$_{0.063}$	46.3	2138.1	27.7	135.2	90.2	26.4	82.0	59.8	1.05	1.65	1.51
AILM$_0$ × CLP$_{0.125}$	46.7	2097.9	27.7	130.9	87.6	26.0	80.3	58.6	1.07	1.63	1.50
AILM$_{0.063}$ × CLP$_0$	46.7	2052.1	27.3	126.6	84.9	25.9	78.3	57.3	1.06	1.62	1.48
AILM$_{0.063}$ × CLP$_{0.063}$	45.9	2009.2	26.5	122.9	82.3	25.4	76.6	56.1	1.05	1.61	1.47
AILM$_{0.063}$ × CLP$_{0.125}$	46.8	1958.2	25.5	118.5	79.3	25.1	74.3	54.6	1.02	1.60	1.45
AILM$_{0.125}$ × CLP$_0$	45.9	1894.9	24.7	113.7	76.1	24.9	71.5	52.8	0.99	1.59	1.44
AILM$_{0.125}$ × CLP$_{0.063}$	46.5	1813.6	23.8	106.5	71.4	24.4	67.9	50.5	0.98	1.57	1.41
AILM$_{0.125}$ × CLP$_{0.125}$	45.9	1729.3	22.9	99.5	66.8	23.9	64.3	48.1	0.96	1.55	1.39
SEM[2]	0.11	24.90	0.32	2.11	1.38	0.16	1.09	0.71	0.01	0.01	0.01
Significance[3]											
AILM	NS	***	***	***	***	***	***	***	***	**	***
CLP	NS	*	NS	***	***	*	NS	*	NS	NS	NS
AILM × CLP	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	*

[1] AILM$_0$ = AILM 0% of the basal diet, AILM$_{0.063}$ = AILM 0.063% of the basal diet, AILM$_{0.125}$ = AILM 0.125% of the basal diet, CLP$_0$ = CLP 0% of the basal diet, CLP$_{0.063}$ = CLP 0.063% of the basal diet, CLP$_{0.125}$ = CLP 0.125% of the basal diet;

[2] SEM = Standard error of the means;

[3] NS = Non-significant ($p>0.05$),* = Significant ($p<0.05$),** = Significant ($p<0.01$), *** = Significant ($p<0.001$);

[a-b] Means bearing different superscripts in a column differ ($p<0.05$).

Table 4. Carcass characteristics of the broiler birds fed diet supplemented with fish oil, *Azadirachta indica* leaf meal (AILM) and *Curcuma longa* powder (CLP) in different proportions

Treatment factors[1]	Relative weight of different carcass components as % live weight[2]												
	DP[2]	BRW[3]	BKW[4]	TW[5]	DW[6]	SW[7]	HW[8]	NW[9]	WW[10]	LVW[11]	AFW[12]	BW[13]	SPW[14]
AILM													
0	59.0	17.6	12.2	10.2	8.57	4.63	2.63	2.60	5.27	2.83	1.33	0.11	0.16
0.063	57.1	15.5	12.1	10.7	9.49	5.25	2.81	2.69	5.14	2.41	0.89	0.09	0.20
0.125	57.4	16.3	12.0	10.1	9.10	5.01	2.84	2.65	5.28	2.49	1.26	0.09	0.18
CLP													
0	58.5	16.8	12.0	10.4	9.30	5.09	2.77	2.55	5.18	2.79	1.22	0.10	0.21
0.063	57.3	16.1	12.0	10.3	8.89	4.88	2.76	2.66	5.25	2.65	1.13	0.10	0.15
0.125	57.8	16.4	12.3	10.3	8.96	4.93	2.74	2.73	5.26	2.30	1.13	0.10	0.18
AILM × CLP													
AILM$_{0}$ × CLP$_{0}$	57.6	17.2	11.3	10.0	8.90	4.58	2.50	2.61	5.13	3.25	1.47	0.12	0.21
AILM$_{0}$ × CLP$_{0.063}$	58.3	16.6	12.3	10.5	8.44	4.90	2.82	2.60	5.14	3.04	1.46	0.12	0.15
AILM$_{0}$ × CLP$_{0.125}$	61.1	19.0	13.0	10.2	8.37	4.42	2.57	2.60	5.53	2.21	1.07	0.11	0.13
AILM$_{0.063}$ × CLP$_{0}$	59.0	15.8	12.9	10.7	9.74	5.47	2.81	2.57	5.62	2.32	0.80	0.06	0.22
AILM$_{0.063}$ × CLP$_{0.063}$	57.1	15.8	11.9	10.5	9.46	5.10	2.73	2.85	5.22	2.69	0.61	0.09	0.18
AILM$_{0.063}$ × CLP$_{0.125}$	55.2	14.9	11.6	10.9	9.27	5.20	2.88	2.66	4.59	2.24	1.26	0.12	0.21
AILM$_{0.125}$ × CLP$_{0}$	58.9	17.5	11.9	10.6	9.26	5.22	3.00	2.48	4.80	2.81	1.39	0.13	0.22
AILM$_{0.125}$ × CLP$_{0.063}$	56.5	16.0	11.7	9.83	8.78	4.64	2.74	2.53	5.39	2.23	1.32	0.09	0.12
AILM$_{0.125}$ × CLP$_{0.125}$	57.0	15.4	12.4	9.80	9.26	5.19	2.77	2.94	5.65	2.45	1.07	0.06	0.21
SEM[2]	0.50	0.38	0.20	0.11	0.13	0.10	0.04	0.05	0.10	0.12	0.14	0.01	0.01
Significance[3]													
AILM	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
CLP	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
AILM × CLP	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] AILM$_{0}$ = AILM 0% of the basal diet, AILM$_{0.063}$ = AILM 0.063% of the basal diet, AILM$_{0.125}$ = AILM 0.125% of the basal diet, CLP$_{0}$ = CLP 0% of the basal diet, CLP$_{0.063}$ = CLP 0.063% of the basal diet, CLP$_{0.125}$ = CLP 0.125% of the basal diet;

[2] DP=Dressing percentage,[3] BRW=Breast weight,[4] BKW=Back weight,[5] TW=Thigh weight,[6] DW=Drumstick weight,[7] SW=Shank weight,[8] WW=Wing weight,[9] NW=Neck weight,[10] LW=Liver weight,[11] GW=Gizzard weight,[12] PW=Proventriculus weight, [13] AFW=Abdominal fat weight, BW = Bursa weight, SPW = Spleen weight;

[15] SEM = Standard error of the means;

[16] NS = Non-significant ($p>0.05$), * = Significant ($p<0.05$);

[a-b] Means bearing different superscripts in a column differ ($p<0.05$).

Table 5. Cardio-pulmonary morphometry of the broiler birds fed diet supplemented with fish oil, *Azadirachta indica* leaf meal (AILM) and *Curcuma longa* powder (CLP) in different proportions

Treatment factors[1]	Cardio-pulmonary morphometry													
	HW[2]	HWL[3]	LW[4]	LWL[5]	RV[6]	RVH[7]	RVD[8]	RVT[9]	LV[10]	LVH[11]	LVD[12]	LVT[13]	TV[14]	RV:TV[15]
AILM														
0	10.55	0.49	10.40	0.49	0.47	4.50	1.22	0.18	1.39	13.24	0.98	0.74	1.86	0.25
0.063	9.33	0.48	9.60	0.49	0.56	6.15	1.16	0.14	1.31	14.22	0.94	0.68	1.88	0.30
0.125	9.66	0.48	9.80	0.49	0.54	5.67	1.08	0.18	1.31	13.78	0.96	0.70	1.85	0.29
CLP														
0	9.37	0.47	9.60	0.48	0.46	5.14	1.12	0.14	1.27	14.02	0.96	0.68	1.74	0.27
0.063	10.31	0.52	9.80	0.49	0.52	5.06	1.22	0.22	1.37	13.35	0.96	0.72	1.89	0.27
0.125	9.87	0.46	10.40	0.49	0.59	6.11	1.12	0.14	1.36	13.86	0.96	0.72	1.95	0.30
AILM × CLP														
AILM$_0$ × CLP$_0$	10.45	0.50	11.40	0.55	0.44	4.29	1.20	0.18	1.33	13.03	1.02	0.78	1.77	0.25
AILM$_0$ × CLP$_{0.063}$	10.61	0.53	10.20	0.51	0.49	4.64	1.26	0.24	1.42	13.36	1.02	0.66	1.91	0.26
AILM$_0$ × CLP$_{0.125}$	10.60	0.45	9.60	0.41	0.49	4.59	1.20	0.12	1.41	13.33	0.90	0.78	1.90	0.26
AILM$_{0.063}$ × CLP$_0$	9.48	0.49	10.20	0.52	0.41	4.47	1.20	0.12	1.27	13.76	0.96	0.66	1.69	0.25
AILM$_{0.063}$ × CLP$_{0.063}$	9.72	0.49	9.00	0.46	0.57	5.95	1.20	0.18	1.36	14.05	0.96	0.72	1.93	0.29
AILM$_{0.063}$ × CLP$_{0.125}$	8.81	0.45	9.60	0.49	0.71	8.04	1.08	0.12	1.31	14.85	0.90	0.66	2.02	0.35
AILM$_{0.125}$ × CLP$_0$	8.18	0.43	7.20	0.37	0.54	6.68	0.96	0.12	1.22	15.28	0.90	0.6	1.76	0.31
AILM$_{0.125}$ × CLP$_{0.063}$	10.60	0.53	10.20	0.52	0.49	4.61	1.20	0.24	1.34	12.66	0.90	0.78	1.83	0.27
AILM$_{0.125}$ × CLP$_{0.125}$	10.20	0.49	12.00	0.58	0.58	5.71	1.08	0.18	1.37	13.41	1.08	0.72	1.95	0.30
SEM[2]	0.31	0.01	0.45	0.02	0.02	0.32	0.03	0.01	0.02	0.36	0.02	0.03	0.03	0.01
Significance[3]														
AILM	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*	NS	NS	*
CLP	NS	NS	NS	NS	**	NS	NS	NS	*	NS	NS	NS	***	NS
AILM × CLP	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]AILM$_0$ = AILM 0% of the basal diet, AILM$_{0.063}$ = AILM 0.063% of the basal diet, AILM$_{0.125}$ = AILM 0.125% of the basal diet, CLP$_0$ = CLP 0% of the basal diet, CLP$_{0.063}$ = CLP 0.063% of the basal diet, CLP$_{0.125}$ = CLP 0.125% of the basal diet;

[2]HW = Heart weight (g),[3]HWL = Heart weight (% live weight); [4]LW = Lung weight (g),[5]LWL = Lung weight (% live weight), [6]RV = Right ventricle weight (g),[7]RVH = Right ventricle weight (% heart weight), [8]RVD = Right ventricle diameter (mm),[9]RVT = Right ventricle thickness (mm),[10]LV = Left ventricle weight (g),[11]LVH = Left ventricle weight (% heart weight),[12]LVD = Left ventricle diameter, [13]LVT = Left ventricle thickness (mm), [14]TV = Total ventricle weight (g), [15]RV:TV = Right ventricle weight: total ventricle weight;

[16]SEM = Standard error of the means;

[17]NS = Non-significant ($p>0.05$),* = Significant ($p<0.05$),** = Significant ($p<0.01$), *** = Significant ($p<0.001$);

[a-b]Means bearing different superscripts in a column differ ($p<0.05$).

Table 6. Blood glucose (GL, mg/dl), serum glutamic oxaloacetic transaminase (SGOT, U/L), serum glutamic pyruvic transaminase (SGPT, U/L), uric acid (UA, mg/dl), creatinine (CRT, mg/dl), serum albumin (SA, mg/dl), serum total protein (TP, g/L), serum triglyceride (TG, mg/dl), low density lipoprotein (LDL, mg/dl), high density lipoprotein (HDL, mg/dl) and total cholesterol (TC, mg/dl) of the broiler birds fed diets supplemented with fish oil, *Azadirachta indica* leaf meal (AILM) and *Curcuma longa* powder (CLP) in different proportions

	Hemato-biochemical indices										
Treatment factors[1]	GL (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	UA (mg/dl)	CRT (mg/dl)	SA (g/L)	TP (g/L)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	TC (mg/dl)
AILM											
0	250.9	249.3	13.6	2.33	0.32	12.1	30.2	86.1	25.5	89.7	130.3
0.063	257.5	235.1	11.6	2.27	0.30	10.9	27.9	76.9	17.2	84.9	112.0
0.125	271.8	248.6	14.0	2.12	0.32	11.5	30.4	70.4	29.5	82.4	127.8
CLP											
0	252.9	261.1	13.6	2.27	0.30	11.9	29.8	80.5	19.2	87.1	118.1
0.063	270.6	232.6	13.1	2.38	0.33	11.6	29.8	72.5	21.5	84.3	118.3
0.125	256.7	239.3	12.6	2.07	0.30	11.1	29.0	80.3	31.5	85.6	133.8
AILM × CLP											
AILM$_0$ × CLP$_0$	264.9	276.6	16.5	2.75	0.35	12.9	32.6	84.3	23.3	89.6	128.5
AILM$_0$ × CLP$_{0.063}$	227.2	235.5	12.8	2.40	0.30	12.4	30.6	86.1	28.6	81.1	125.8
AILM$_0$ × CLP$_{0.125}$	260.5	235.9	11.4	1.85	0.30	11.2	27.6	87.8	24.7	98.6	136.7
AILM$_{0.063}$ × CLP$_0$	239.9	230.8	9.50	1.80	0.25	11.0	27.7	76.5	15.5	91.3	111.8
AILM$_{0.063}$ × CLP$_{0.063}$	268.1	241.2	12.9	2.80	0.35	11.7	27.5	64.5	17.6	82.4	111.1
AILM$_{0.063}$ × CLP$_{0.125}$	264.4	233.3	12.6	2.20	0.30	10.2	28.6	89.6	18.6	81.2	113.2
AILM$_{0.125}$ × CLP$_0$	254.0	275.8	14.9	2.25	0.30	11.7	29.1	80.7	18.9	80.5	113.9
AILM$_{0.125}$ × CLP$_{0.063}$	316.4	221.2	13.6	1.95	0.35	10.9	31.3	66.9	18.4	89.6	118.0
AILM$_{0.125}$ × CLP$_{0.125}$	245.1	248.8	13.7	2.15	0.30	11.9	30.9	63.6	51.1	77.1	151.6
SEM[2]	8.11	7.94	0.60	0.10	0.01	0.36	0.65	4.59	3.20	3.37	5.09
Significance[3]											
AILM	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CLP	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
AILM × CLP	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] AILM$_0$ = AILM 0% of the basal diet, AILM$_{0.063}$ = AILM 0.063% of the basal diet, AILM$_{0.125}$ = AILM 0.125% of the basal diet, CLP$_0$ = CLP 0% of the basal diet, CLP$_{0.063}$ = CLP 0.063% of the basal diet, CLP$_{0.125}$ = CLP 0.125% of the basal diet;

[2] PW = Proventriculus weight (g), GW = Gizzard weight, L = Length (cm), W = Weight (g), D = Diameter (cm);

[15] SEM = Standard error of the means;

[16] NS = Non-significant ($p>0.05$), * = Significant ($p<0.05$);

[a-b] Means bearing different superscripts in a column differ ($p<0.05$).

Table 7. Gut morphology of the broiler birds fed diet supplemented with fish oil, *Azadirachta indica* leaf meal (AILM) and *Curcuma longa* powder (CLP) in different proportions

Treatment factors[1]	Gut morphology[2]										
	PW	GW	Duodenum			Jejunum			Ileum		
			L	W	D	L	W	D	L	W	D
AILM											
0	11.4	64.0	40.5	16.7	3.26	98.0	55.4	3.14	61.4	27.1	3.04
0.063	12.2	63.0	36.1	15.0	3.28	101	50.2	3.16	66.2	30.9	3.16
0.125	11.2	57.6	37.2	14.7	2.58	92.9	51.9	3.16	69.0	29.9	3.20
CLP											
0	12.0	62.8	36.9	15.3	2.96	96.1	52.9	3.02	58.2	24.8	2.96
0.063	11.2	60.8	40.5	17.5	3.22	96.2	54.9	3.22	70.8	34.3	3.28
0.125	11.6	61.0	36.4	13.7	2.94	99.4	49.7	3.22	67.6	28.7	3.16
AILM × CLP											
AILM$_0$ × CLP$_0$	12.0	60.6	39.3	16.6	3.36	97.8	58.0	3.00	57.0	26.2	3.06
AILM$_0$ × CLP$_{0.063}$	11.4	63.6	45.9	19.8	3.60	91.1	55.9	3.42	69.6	32.6	3.06
AILM$_0$ × CLP$_{0.125}$	10.8	67.8	36.3	13.7	2.82	105	52.3	3.00	57.6	22.4	3.00
AILM$_{0.063}$ × CLP$_0$	12.0	64.2	36.0	14.9	3.24	103	46.5	2.52	52.8	18.1	2.70
AILM$_{0.063}$ × CLP$_{0.063}$	13.2	63.0	37.8	15.9	3.00	98.4	54.9	3.30	74.4	43.3	3.60
AILM$_{0.063}$ × CLP$_{0.125}$	11.4	61.8	34.4	14.1	3.60	101	49.4	3.66	71.4	31.4	3.18
AILM$_{0.125}$ × CLP$_0$	12.0	63.6	35.4	14.3	2.28	88.0	54.4	3.54	64.8	30.3	3.12
AILM$_{0.125}$ × CLP$_{0.063}$	9.0	55.8	37.8	16.7	3.06	99.0	54.0	2.94	68.4	27.2	3.18
AILM$_{0.125}$ × CLP$_{0.125}$	12.6	53.4	38.4	13.2	2.40	91.8	47.4	3.00	73.8	32.3	3.30
SEM[2]	0.36	1.20	0.87	0.59	0.15	1.97	1.59	0.11	2.82	2.11	0.12
Significance[3]											
AILM	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
CLP	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
AILM × CLP	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] AILM$_0$ = AILM 0% of the basal diet, AILM$_{0.063}$ = AILM 0.063% of the basal diet, AILM$_{0.125}$ = AILM 0.125% of the basal diet, CLP$_0$ = CLP 0% of the basal diet, CLP$_{0.063}$ = CLP 0.063% of the basal diet, CLP$_{0.125}$ = CLP 0.125% of the basal diet;

[2] PW = Proventriculus weight (g), GW = Gizzard weight, L = Length (cm), W = Weight (g), D = Diameter (cm);

[15] SEM = Standard error of the means;

[16] NS = Non-significant (p>0.05), * = Significant (p<0.05);

[a-b] Means bearing different superscripts in a column differ (p<0.05).

Table 8. Apparent ileal digestibility (AID, %) of the dry matter (DM), organic matter (OM), crude protein (CP), crude fibre (CF), ether extract (EE), nitrogen free extract (NFE), total ash (TA), calcium (Ca) and phosphorus (P) in the broiler birds fed diet supplemented with fish oil, *Azadirachta indica* leaf meal (AILM) and *Curcuma longa* powder (CLP) in different proportions

Treatment factors[1]	AID (%)								
	DM	OM	CP	EE	CF	NFE	TA	Ca	P
AILM									
0	70.6	71.6	69.9	71.2	39.9	72.2	58.3	75.9	69.1
0.063	70.7	71.8	76.0	73.2	42.5	72.2	56.4	77.1	67.6
0.125	70.5	71.5	76.6	74.5	40.0	71.3	56.7	76.8	67.9
CLP									
0	70.5	71.6	73.1	70.3	43.7	72.2	57.0	77.4	67.6
0.063	70.7	71.7	74.7	75.7	40.6	71.4	57.2	75.8	68.3
0.125	70.6	71.6	74.8	72.9	38.1	72.2	57.2	76.5	68.6
AILM × CLP									
$AILM_0 \times CLP_0$	70.1	71.1	68.0	64.6	44.6	72.0	57.8	76.5	67.8
$AILM_0 \times CLP_{0.063}$	70.6	71.4	71.0	73.3	41.0	71.0	60.9	74.6	70.1
$AILM_0 \times CLP_{0.125}$	71.0	72.1	70.8	75.7	34.0	73.7	56.2	76.5	69.3
$AILM_{0.063} \times CLP_0$	70.7	71.9	77.2	72.2	46.1	72.6	55.6	77.8	67.6
$AILM_{0.063} \times CLP_{0.063}$	70.7	71.8	76.6	73.5	40.9	71.9	56.2	75.5	67.6
$AILM_{0.063} \times CLP_{0.125}$	70.7	71.7	74.2	73.8	40.5	72.1	57.4	77.9	67.5
$AILM_{0.125} \times CLP_0$	70.7	71.7	74.2	74.0	40.3	72.0	57.6	77.9	67.4
$AILM_{0.125} \times CLP_{0.063}$	70.6	71.9	76.3	80.2	40.0	71.3	54.4	77.2	67.3
$AILM_{0.125} \times CLP_{0.125}$	70.1	71.1	79.4	69.2	39.7	70.7	58.0	75.2	69.1
SEM[2]	0.14	0.12	0.84	0.99	0.78	0.23	0.43	0.31	0.27
Significance[3]									
AILM	NS	NS	***	NS	NS	NS	NS	NS	NS
CLP	NS	NS	NS	NS	***	NS	NS	NS	NS
AILM × CLP	NS	NS	***	***	***	*	***	*	*

[1] $AILM_0$ = AILM 0% of the basal diet, $AILM_{0.063}$ = AILM 0.063% of the basal diet, $AILM_{0.125}$ = AILM 0.125% of the basal diet, CLP_0 = CLP 0% of the basal diet, $CLP_{0.063}$ = CLP 0.063% of the basal diet, $CLP_{0.125}$ = CLP 0.125% of the basal diet;

[2] PW = Proventriculus weight (g), GW = Gizzard weight, L = Length (cm), W = Weight (g), D = Diameter (cm);

[15] SEM = Standard error of the means;

[16] NS = Non-significant (p>0.05), * = Significant (p<0.05);

[a-b] Means bearing different superscripts in a column differ (p<0.05).

Table 9. Tibia morphometry of the broiler birds fed diet supplemented with fish oil, *Azadirachta indica* leaf meal (AILM) and *Curcuma longa* powder (CLP) in different proportions

Treatment factors[1]	Tibia morphometry							
	Length (cm)	Diameter (cm)	Weight (g)	Weight (% LW)	Length: Diameter	TA	Ca (%)	P (%)
AILM								
0	8.03	0.81	4.46	0.21	9.92	42.5	6.22	9.03
0.063	8.13	0.78	4.50	0.23	10.4	44.2	6.86	11.5
0.125	7.97	0.81	4.44	0.22	9.86	39.6	8.45	8.65
CLP								
0	7.78	0.75	3.88	0.20	10.4	43.0	6.64	10.9
0.063	8.07	0.80	4.38	0.22	10.1	41.8	7.82	8.38
0.125	8.28	0.86	5.14	0.24	9.71	41.4	7.07	9.88
AILM × CLP								
$\text{AILM}_0 \times \text{CLP}_0$	7.75	0.78	3.86	0.19	10.0	43.9	5.65	8.95
$\text{AILM}_0 \times \text{CLP}_{0.063}$	8.05	0.80	4.27	0.22	10.1	43.4	6.45	9.60
$\text{AILM}_0 \times \text{CLP}_{0.125}$	8.30	0.87	5.25	0.23	9.60	40.1	6.55	8.55
$\text{AILM}_{0.063} \times \text{CLP}_0$	7.90	0.74	4.04	0.21	10.7	45.6	7.28	12.9
$\text{AILM}_{0.063} \times \text{CLP}_{0.063}$	8.00	0.76	4.03	0.21	10.6	44.3	6.95	10.6
$\text{AILM}_{0.063} \times \text{CLP}_{0.125}$	8.50	0.86	5.44	0.28	9.98	42.5	6.35	10.9
$\text{AILM}_{0.125} \times \text{CLP}_0$	7.70	0.75	3.76	0.20	10.3	39.6	7.00	10.8
$\text{AILM}_{0.125} \times \text{CLP}_{0.063}$	8.15	0.84	4.85	0.25	9.76	37.8	10.1	4.95
$\text{AILM}_{0.125} \times \text{CLP}_{0.125}$	8.05	0.85	4.73	0.23	9.55	41.5	8.30	10.2
SEM[2]	0.07	0.01	0.15	0.01	0.14	0.61	0.36	0.57
Significance[3]								
AILM	NS	NS	NS	NS	NS	***	**	NS
CLP	***	***	***	*	*	***	NS	NS
AILM × CLP	NS	NS	NS	NS	NS	***	NS	NS

[1] AILM_0 = AILM 0% of the basal diet, $\text{AILM}_{0.063}$ = AILM 0.063% of the basal diet, $\text{AILM}_{0.125}$ = AILM 0.125% of the basal diet, CLP_0 = CLP 0% of the basal diet, $\text{CLP}_{0.063}$ = CLP 0.063% of the basal diet, $\text{CLP}_{0.125}$ = CLP 0.125% of the basal diet;

[2] SEM = Standard error of the means;

[3] NS = Non-significant ($p>0.05$),* = Significant ($p<0.05$), *** = Significant ($p<0.001$);

[a-b] Means bearing different superscripts in a column differ ($p<0.05$).

Table 10. The pH, malonaldehyde concentration (MDA, μmol/100g), crude protein (CP), crude fibre (CF), ether extract (EE), nitrogen free extract (NFE) and total ash (TA) contents in the meat (Pectoralis major) of the broiler birds fed diets supplemented with fish oil, *Azadirachta indica* leaf meal (AILM) and *Curcuma longa* powder (CLP) in different proportions

Treatment factors[1]	Meat characteristics									
	p^H	MDA (μmol/100g)			Proximate components (%)					
		MDA^0	MDA^7	MDA^{14}	Moisture	CP	EE	CF	NFE	TA
AILM										
0	5.62	0.24	1.33	5.00	74.0	75.5	20.4	-	1.60	1.32
0.063	5.58	0.19	1.44	7.49	74.7	74.7	21.6	-	1.36	1.18
0.125	5.77	0.57	2.57	5.94	73.6	73.8	22.6	-	1.11	0.95
CLP										
0	5.61	0.37	1.69	6.84	74.8	75.2	20.9	-	1.42	1.18
0.063	5.72	0.36	2.09	6.28	74.2	74.7	21.5	-	1.34	1.16
0.125	5.63	0.27	1.55	5.31	73.4	74.2	22.2	-	1.30	1.10
AILM × CLP										
AILM$_0$ × CLP$_0$	5.73	0.23	0.72	4.62	74.9	76.1	19.6	-	1.74	1.43
AILM$_0$ × CLP$_{0.063}$	5.78	0.30	2.18	8.25	74.1	75.6	20.5	-	1.49	1.32
AILM$_0$ × CLP$_{0.125}$	5.37	0.19	1.09	2.14	73.1	74.8	21.2	-	1.56	1.20
AILM$_{0.063}$ × CLP$_0$	5.42	0.15	1.54	5.41	75.0	74.8	21.5	-	1.34	1.15
AILM$_{0.063}$ × CLP$_{0.063}$	5.63	0.23	1.43	6.04	74.8	74.6	21.4	-	1.48	1.16
AILM$_{0.063}$ × CLP$_{0.125}$	5.69	0.19	1.35	11.0	74.3	74.9	21.9	-	1.28	1.22
AILM$_{0.125}$ × CLP$_0$	5.69	0.72	2.82	10.5	74.4	74.8	21.5	-	1.20	0.97
AILM$_{0.125}$ × CLP$_{0.063}$	5.77	0.57	2.67	4.54	73.6	73.9	22.6	-	1.05	1.01
AILM$_{0.125}$ × CLP$_{0.125}$	5.84	0.42	2.22	2.78	72.8	72.8	23.6	-	1.08	0.88
SEM[2]	0.05	0.05	0.17	0.73	0.19	0.24	0.27	-	0.05	0.04
Significance[3]										
AILM	NS	**	***	NS		NS	*	-	***	*
CLP	NS	NS	NS	NS		NS	*	-	NS	*
AILM × CLP	NS	NS	NS	NS		NS	NS	-	NS	NS

[1] AILM$_0$ = AILM 0% of the basal diet, AILM$_{0.063}$ = AILM 0.063% of the basal diet, AILM$_{0.125}$ = AILM 0.125% of the basal diet, CLP$_0$ = CLP 0% of the basal diet, CLP$_{0.063}$ = CLP 0.063% of the basal diet, CLP$_{0.125}$ = CLP 0.125% of the basal diet;

[2] PW = Proventriculus weight (g), GW = Gizzard weight, L = Length (cm), W = Weight (g), D = Diameter (cm);

[15] SEM = Standard error of the means;

[16] NS = Non-significant ($p>0.05$), * = Significant ($p<0.05$);

[a-b] Means bearing different superscripts in a column differ ($p<0.05$).

Table 11. Fatty acid of the breast muscle (Pectoralis major) of the broiler birds fed diets supplemented with fish oil, *Azadirachta indica* leaf meal (AILM) and *Curcuma longa* powder (CLP) in different proportions

Fatty acids	Fatty acid profile of the Pectoralis major muscle of the experimental broiler chicken																		
	AILM			CLP			AILM × CLP									SEM	Significance		
	0	0.063	0.125	0	0.063	0.125	$AILM_0$ × CLP_0	$AILM_0$ × $CLP_{0.063}$	$AILM_0$ × $CLP_{0.125}$	$AILM_{0.063}$ × CLP_0	$AILM_{0.063}$ × $CLP_{0.063}$	$AILM_{0.063}$ × $CLP_{0.125}$	$AILM_{0.125}$ × CLP_0	$AILM_{0.125}$ × $CLP_{0.063}$	$AILM_{0.125}$ × $CLP_{0.125}$		A	C	A×C
C4:0	35.1	27.6	14.8	26.3	24.5	26.7	35.4	36.5	33.5	23.0	32.3	27.5	20.5	4.55	19.3	2.54	***	*	NS
C8:0	0.09	0.02	0.08	0.04	0.04	0.10	0.08	0.13	0.07	0.05	0.00	0.00	0.00	0.00	0.23	0.02	**	***	**
C14:0	0.36	0.40	0.48	0.42	0.40	0.42	0.41	0.31	0.36	0.41	0.35	0.44	0.44	0.55	0.45	0.02	NS	NS	NS
C15:0	2.11	1.27	1.40	1.82	1.63	1.32	3.37	1.62	1.35	0.98	1.44	1.38	1.11	1.84	1.24	0.19	NS	NS	NS
C16:0	14.6	16.4	19.7	16.4	16.6	17.8	13.9	13.4	16.5	17.0	14.4	17.9	18.1	22.1	18.9	0.72	**	NS	NS
C17:0	2.59	2.17	3.30	2.35	2.31	3.40	2.16	1.99	3.62	2.32	0.81	3.39	2.57	4.15	3.18	0.27	*	NS	NS
C18:0	4.69	5.00	5.92	5.08	5.49	5.03	4.87	4.45	4.76	4.91	5.21	4.89	5.47	6.82	5.45	0.17	***	**	**
C20:0	7.63	17.0	5.31	11.5	11.6	6.90	0.00	15.8	7.04	18.5	18.8	13.7	15.9	0.00	0.00	1.98	NS	NS	**
C21:0	0.36	0.56	0.44	0.51	0.48	0.37	0.38	0.40	0.30	0.59	0.62	0.47	0.56	0.42	0.33	0.03	NS	NS	NS
C22:0	0.72	0.09	0.31	0.74	0.14	0.24	1.96	0.00	0.20	0.05	0.00	0.22	0.22	0.42	0.30	0.19	NS	NS	NS
C18:3, *c*-9,12,15, ω-3	0.20	0.23	0.36	0.26	0.27	0.26	0.18	0.20	0.23	0.25	0.20	0.25	0.34	0.43	0.30	0.02	***	***	**
C21:5, *c*-5,8,11,14,17, ω-3	0.07	0.11	0.10	0.08	0.04	0.15	0.00	0.06	0.15	0.08	0.07	0.17	0.16	0.00	0.14	0.02	NS	**	NS
C22:6, *c*-4,7,10,13,16,19, ω-3	0.61	0.63	0.51	0.52	0.65	0.58	0.53	0.68	0.63	0.43	0.82	0.65	0.61	0.45	0.47	0.03	NS	NS	NS
18:2, *c*-9,12, ω-6	5.07	0.05	9.11	3.60	5.29	5.34	10.6	0.00	4.60	0.08	0.06	0.00	0.11	15.8	11.4	1.48	*	NS	**
C20:2, *c*-11,14, ω-6	0.20	0.22	0.18	0.20	0.26	0.14	0.19	0.23	0.18	0.22	0.33	0.11	0.19	0.21	0.14	0.02	NS	NS	NS
C20:4, *c*-5,8,11,14, ω-6	3.43	3.09	3.04	3.00	3.74	2.83	3.99	3.44	2.87	2.38	4.15	2.74	2.63	3.64	2.86	0.17	NS	NS	NS
C16:1, ω-7	0.25	0.35	0.47	0.32	0.36	0.39	0.20	0.26	0.28	0.35	0.33	0.37	0.40	0.48	0.52	0.03	*	NS	NS
C17:1, *c*-10, ω-7	1.77	0.48	0.43	1.73	0.57	0.38	4.38	0.60	0.34	0.41	0.60	0.43	0.40	0.53	0.38	0.43	NS	NS	NS
C22:1, ω-9	0.52	0.48	0.55	0.49	0.57	0.50	0.55	0.52	0.50	0.40	0.53	0.52	0.51	0.67	0.48	0.02	NS	NS	NS
C24:1, *c*-15, ω-9	0.65	0.67	0.55	0.60	0.77	0.50	0.75	0.70	0.51	0.51	0.96	0.53	0.55	0.66	0.45	0.04	NS	*	*
C18:1, *c*-9, ω-9	18.9	23.0	32.9	24.0	24.3	26.5	16.0	18.6	22.0	26.9	17.9	24.3	29.1	36.3	33.4	1.71	***	NS	NS
C20:1, *c*-11, ω-9	0.07	0.17	0.11	0.15	0.08	0.13	0.09	0.06	0.07	0.18	0.17	0.17	0.18	0.00	0.14	0.02	*	*	*
∑ω-3	0.89	0.97	0.97	0.86	0.97	0.99	0.71	0.94	1.01	0.76	1.08	1.06	1.11	0.88	0.91	0.04	NS	NS	NS
∑ω-6	8.70	3.36	12.3	6.79	9.29	8.31	14.8	3.67	7.66	2.68	4.54	2.85	2.93	19.7	14.4	1.53	*	*	**
∑ω-7	2.02	0.83	0.90	2.05	0.93	0.77	4.58	0.86	0.62	0.77	0.92	0.80	0.80	1.01	0.90	0.42	NS	NS	NS
∑ω-9	20.1	24.3	34.1	25.2	25.7	27.7	17.4	19.9	23.1	27.9	19.6	25.5	30.3	37.6	34.5	1.69	***	NS	NS

∑ω-6:∑ω-3	10.7	3.49	13.6	8.99	10.2	8.62	20.8	3.91	7.31	3.54	4.23	2.69	2.64	22.3	15.9	1.88	NS	NS	***
∑ω-3:∑PUFA	0.13	0.23	0.13	0.18	0.15	0.16	0.05	0.21	0.15	0.22	0.19	0.27	0.27	0.04	0.06	0.02	NS	NS	***
∑ω-6:∑PUFA	0.87	0.77	0.87	0.82	0.85	0.84	0.95	0.79	0.85	0.78	0.81	0.73	0.73	0.96	0.94	0.02	NS	NS	***
∑ω-9:∑MUFA	0.91	0.97	0.97	0.92	0.96	0.97	0.81	0.96	0.97	0.97	0.95	0.97	0.97	0.97	0.97	0.02	NS	NS	NS
C18:1, c-9, ω-9:∑MUFA	0.86	0.91	0.94	0.87	0.90	0.93	0.75	0.89	0.93	0.93	0.87	0.92	0.93	0.94	0.94	0.02	*	NS	NS
C18:1, c-9, ω-9:∑UFA	0.62	0.77	0.70	0.70	0.67	0.72	0.43	0.73	0.69	0.83	0.68	0.80	0.83	0.61	0.66	0.03	NS	NS	**
∑SFA	68.3	70.5	51.7	65.1	63.1	62.3	62.5	74.7	67.7	67.9	73.9	69.8	64.9	40.9	49.3	2.67	***	*	**
∑UFA	31.7	29.5	48.3	34.9	36.9	37.7	37.46	25.4	32.3	32.1	26.1	30.2	35.1	59.1	50.7	2.67	***	*	**
∑MUFA	22.1	25.2	35.0	27.3	26.6	28.4	21.97	20.8	23.7	28.7	20.5	26.3	31.1	38.6	35.4	1.61	**	NS	NS
∑PUFA	9.59	4.32	13.3	7.65	10.3	9.30	15.49	4.61	8.66	3.44	5.63	3.91	4.04	20.5	15.3	1.52	*	*	**
∑UFA:∑SFA	0.48	0.42	1.01	0.54	0.72	0.65	0.60	0.34	0.48	0.48	0.35	0.43	0.54	1.45	1.04	0.09	***	***	***
∑MUFA:∑UFA	0.71	0.85	0.75	0.79	0.75	0.77	0.58	0.81	0.74	0.89	0.78	0.87	0.89	0.65	0.70	0.03	NS	NS	**
∑MUFA:∑SFA	0.33	0.36	0.72	0.42	0.50	0.48	0.35	0.28	0.35	0.43	0.28	0.38	0.48	0.95	0.72	0.05	***	NS	**
∑PUFA:∑UFA	0.29	0.15	0.25	0.21	0.25	0.23	0.42	0.19	0.26	0.11	0.22	0.13	0.11	0.35	0.30	0.03	NS	NS	**
∑PUFA:∑SFA	0.15	0.06	0.29	0.12	0.21	0.17	0.25	0.06	0.13	0.05	0.08	0.06	0.06	0.51	0.31	0.04	NS	NS	***
∑PUFA:∑MUFA	0.44	0.18	0.36	0.33	0.35	0.32	0.73	0.24	0.37	0.12	0.28	0.15	0.13	0.53	0.43	0.05	NS	NS	**

[1] Azadirachta 0 = AILM 0% of basal diet, Azadirachta 0.063 = AILM 0.063% of basal diet, Azadirachta 0.125 = AILM 0.125% of basal diet, Curcuma 0 = CLM 0% of basal diet, Curcuma 0.063 = CLM 0.063% of basal diet, Curcuma 0.125 = CLM 0.125% of basal diet;

[2] PW = Proventriculus weight (g), GW = Gizzard weight, L = Length (cm), W = Weight (g), D = Diameter (cm);

[15] SEM = Standard error of the means;

[16] NS = Non-significant ($p>0.05$), * = Significant ($p<0.05$), ** = Significant ($p<0.01$), *** = Significant ($p<0.001$);

[a-b] Means bearing different superscripts in a column differ ($p<0.05$).

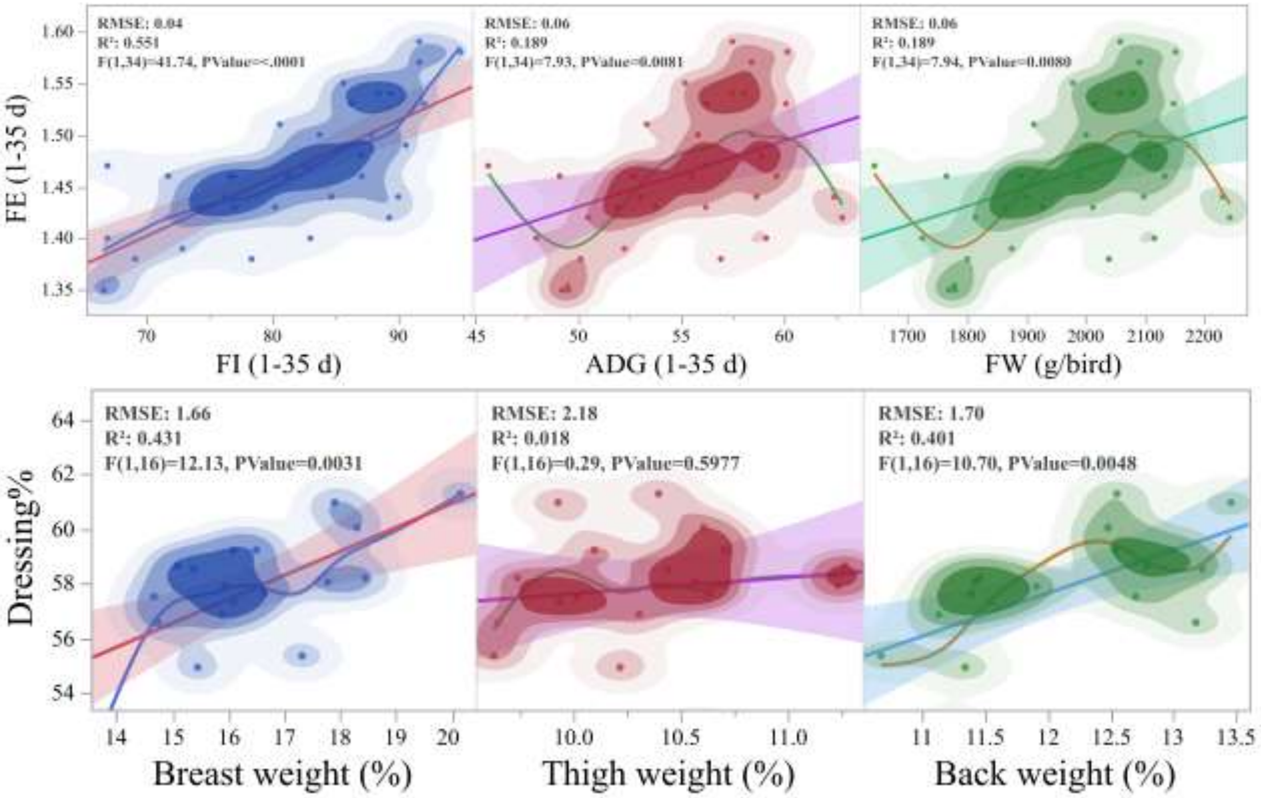

Figure 1. Bivariate linear regression analysis with locally weighted scatterplot smoothing, contour plot and line of fit (95% CI) showing effects of feed intake (FI), average daily gain (ADG) and final live weight (FLW) on feed efficiency (FE); association of breast weight (%), thigh weight (%) and back weight (%) with dressing% of the broiler chicken

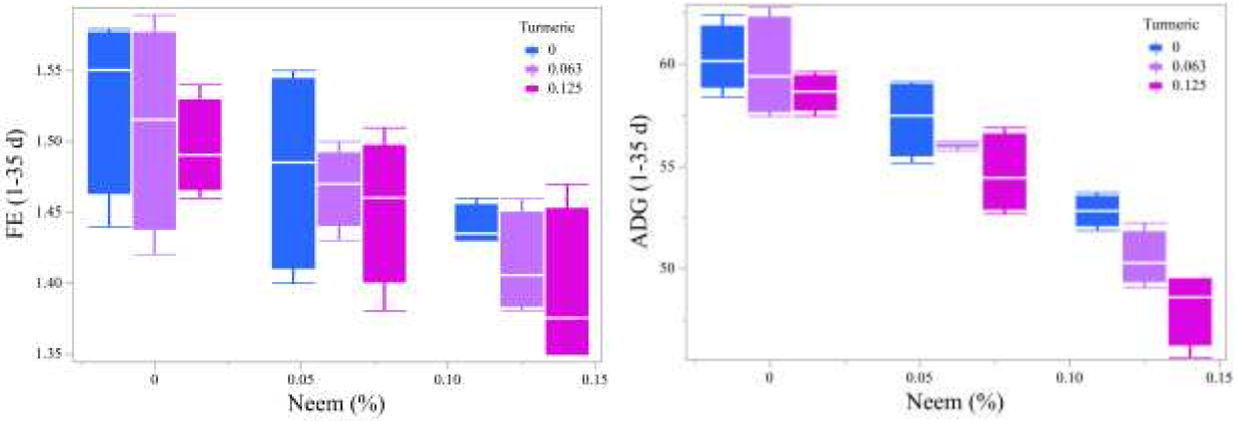

Figure 2. Boxplot showing effects of *Azadirachta indica* (Neem) and *Curcuma longa* (Turmeric) on feed efficiency (FE) of the broiler chicken plotted

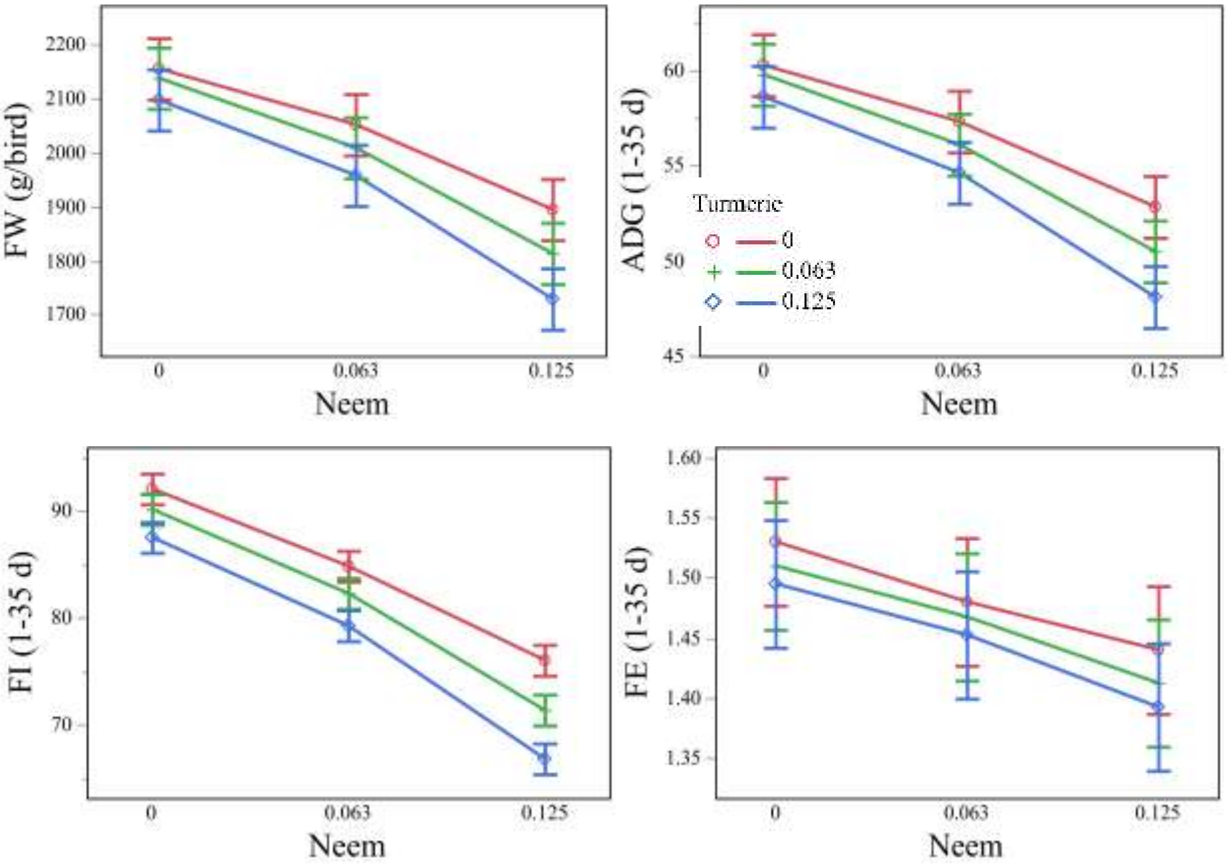

Figure 3. Least square means of the interaction effects of *Azadirachta indica* (Neem) and *Curcuma longa* (Turmeric) on final weight (FW), average daily gain (ADG), feed intake (FI) and feed efficiency (FE) of the broiler chicken

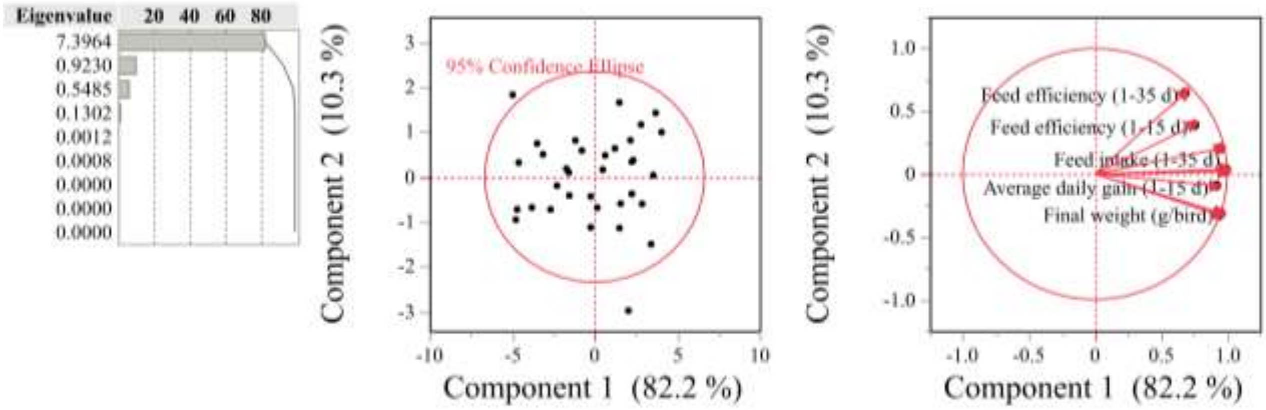

Figure 4. Principal component analysis showing effects of *Azadirachta indica* (Neem) and *Curcuma longa* (Turmeric) on dimensionality and latent trends of the performance parameters of the broiler chicken plotted on 'x' as component 1 (76.3%) and 'y' as component 2 (15.3%)

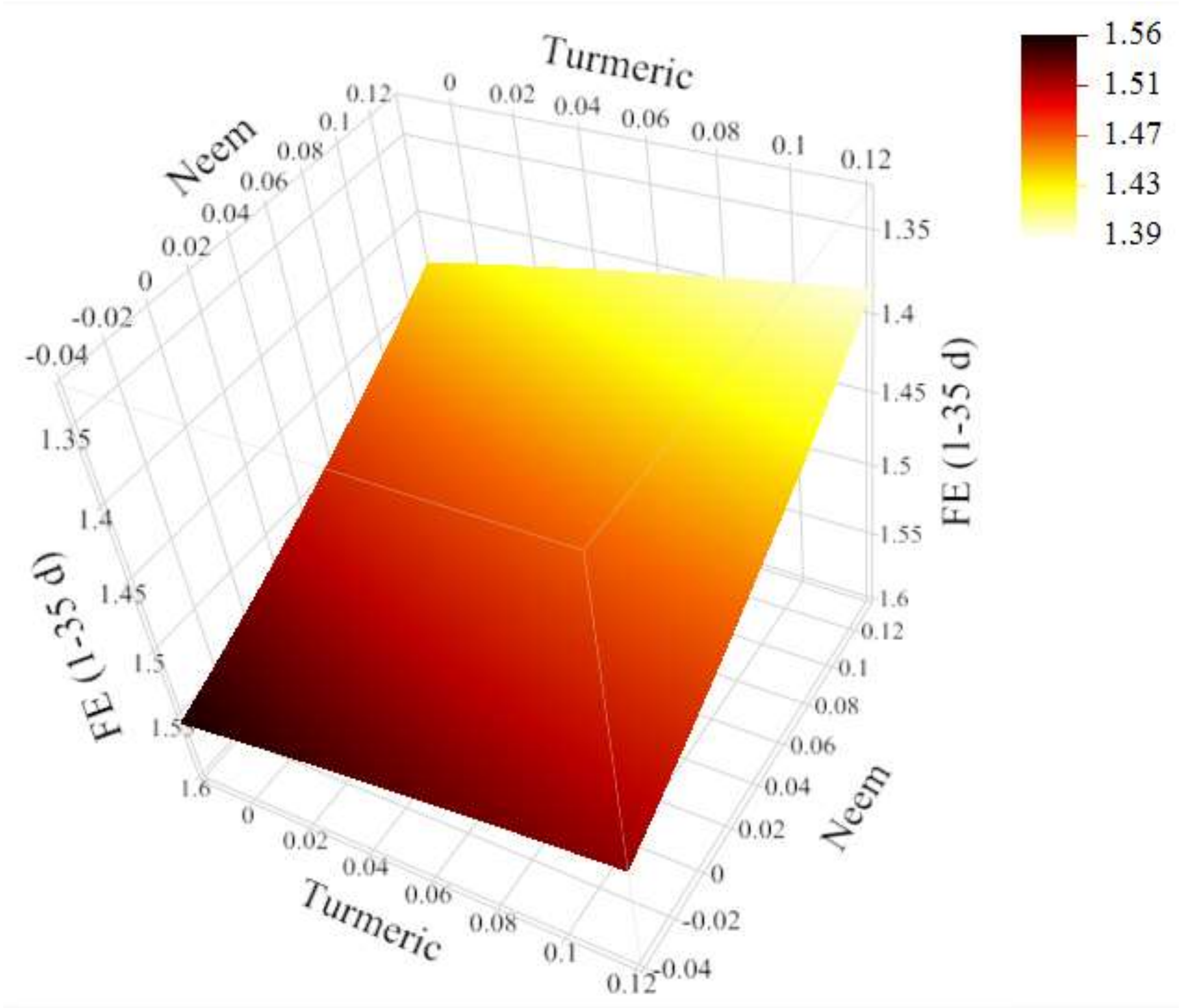

Figure 5. A response surface model with central composite design showing desirable zone of feed efficiency optimized by factorial combinations of *Azadirachta indica* (Neem) and *Curcuma longa* (Turmeric) in broiler chicken

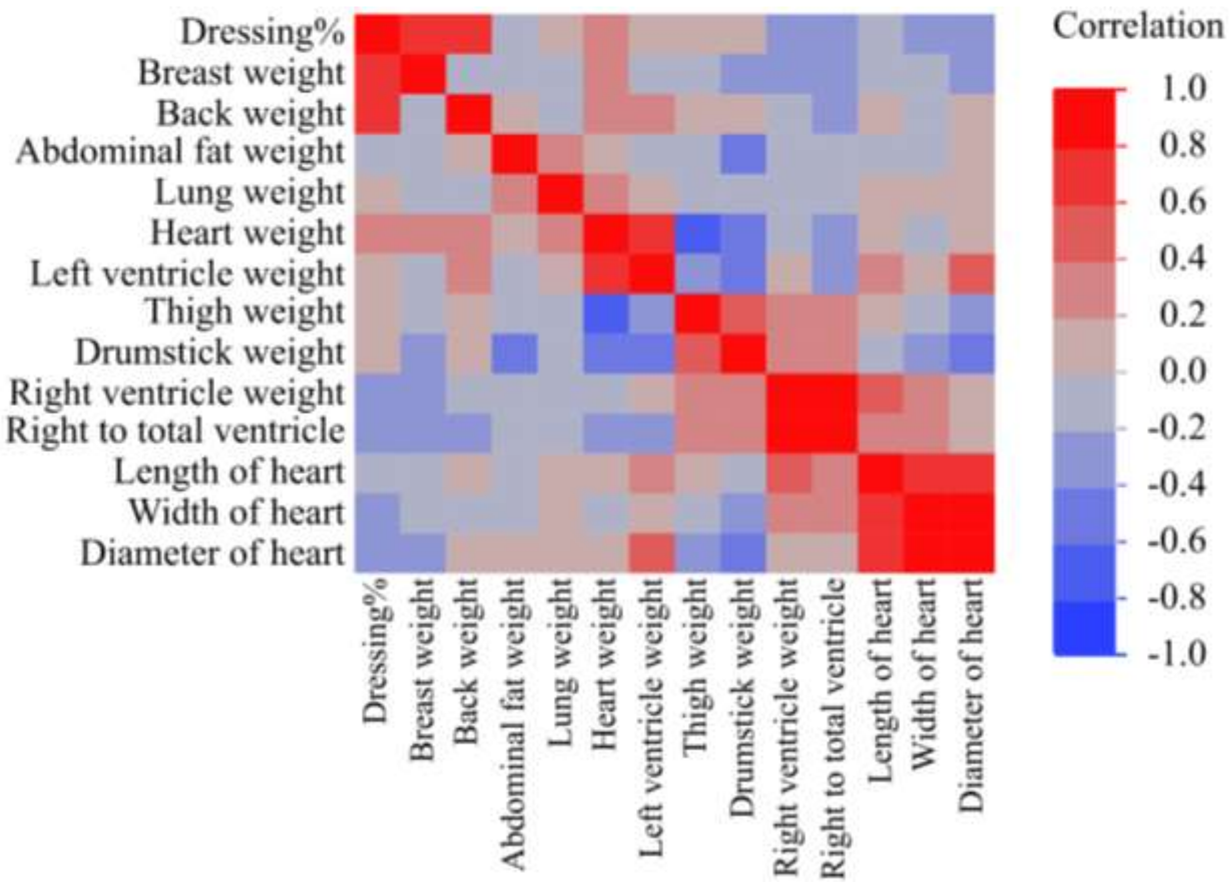

Figure 6. Heatmap showing multiple associations of carcass characteristics and cardio-pulmonary morphometry of the broiler chicken fed diet supplemented with various levels of *Azadirachta indica* (Neem) and *Curcuma longa* (Turmeric)

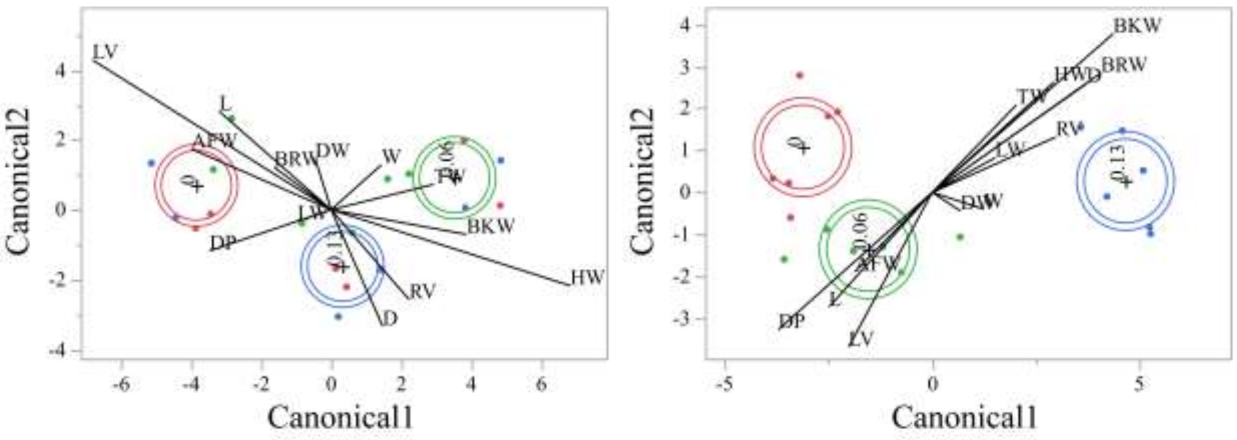

Figure 7. Multiple canonical discriminant analysis showing partitioning effects of *Azadirachta indica* (Neem, top) and *Curcuma longa* (Turmeric, bottom) of performance parameter, carcass traits and tibia morphometry of the broiler chicken

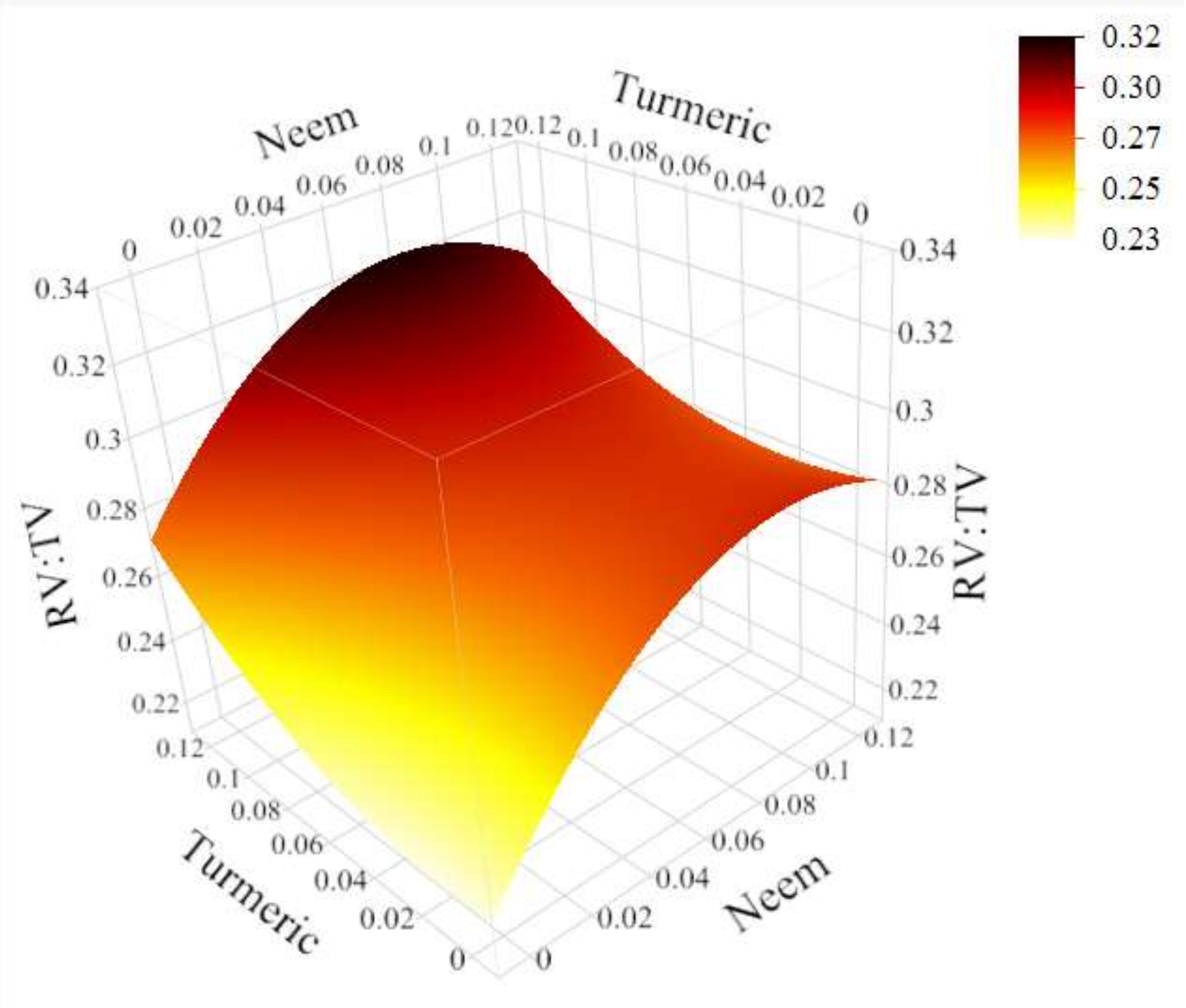

Figure 8. A response surface model with central composite design showing desirable zone of right to total ventricle ratio (RV: TV) optimized by factorial combinations of *Azadirachta indica* (Neem) and *Curcuma longa* (Turmeric) in broiler chicken