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PAPER



## Pomegranate (*Punica granatum* L.) peel powder meal supplementation in broilers: effect on growth performance, digestibility, carcase and organ weights, serum and some meat antioxidant enzyme biomarkers

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### ABSTRACT

This study determined the dietary effects of pomegranate peel powder meal (PPPM) supplementation on growth performance, digestibility, carcase and organ weights, serum, and some meat antioxidant enzyme indices of Cobb 500 birds. Birds ( $n = 432$ ) were randomly assigned to six experimental groups of four replicates each and were fed either diets supplemented with 2, 4, 6 and 8 g/kg of PPPM (PPPM<sub>2</sub>, PPPM<sub>4</sub>, PPPM<sub>6</sub> and PPPM<sub>8</sub>), 0% additives (Negative control; NEGCON), or with  $\alpha$ -Tocopherol acetate at 200 g/tonne (Positive control; POSCON). The diet  $\times$  week interaction effect on feed intake (FI) and feed conversion ratio (FCR) showed that the POSCON diet promoted a better FI and FCR at week 3. However, the FCR of the POSCON birds was comparable to that of the NEGCON, PPPM<sub>4</sub> and PPPM<sub>6</sub> birds. The average final body weight and average daily weight gain was highest in the PPPM<sub>2</sub> and PPPM<sub>4</sub> birds, whereas, PPPM<sub>2</sub> birds had improved FCR and protein efficiency ratio compared with the POSCON birds. High weight was highest in the PPPM<sub>4</sub> group, whereas PPPM<sub>8</sub> birds had the highest breast weight compared with the POSCON. Birds fed the PPPM<sub>4</sub> diet also had improved nutrient digestibility compared with the POSCON birds. Spleen and gizzard weights were highest in PPPM<sub>4</sub> birds compared with the NEGCON group. The concentration of serum aspartate amino-transferase (AST) was decreased in PPPM<sub>4</sub> birds, whereas meat from PPPM<sub>8</sub> group had the highest catalase enzyme activity. It can be concluded that birds fed 4 g/kg PPPM outperformed the birds that were fed  $\alpha$ -tocopherol supplemented (POSCON) diets in terms of performance, digestibility, carcase and organ indices. The effect of pomegranate peel powder meal supplementation was also pronounced in decreasing the concentration of AST at 4 g/kg and increasing the activity of catalase enzyme at 8 g/kg compared with the POSCON and NEGCON diets.

### HIGHLIGHTS

- Dietary pomegranate peel powder can improve performance in broilers.
- Dietary pomegranate peel powder can enhance the enzyme activity of broiler meat.

### ARTICLE HISTORY

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### KEYWORDS

Performance; digestibility; pomegranate peel; feed additives; broilers

## Introduction

Pomegranate peel is among the spectrum of natural feed additives that has received increased attention over the years as prophylactics and growth enhancers in broiler nutrition. Pomegranate (*Punica granatum* L.) is a highly nutritious natural feed additive with numerous bioactive constituents and potent pharmacological properties (Arendse et al. 2017). Pomegranate is a non-

climateric, deciduous, and ornamental plant that is extensively grown in many parts of the world, including South Africa. The plant is known for its hardiness, and ability to withstand harsh climatic and environmental conditions (Dhinesh and Ramasamy 2016). Pomegranate peel is the inedible portion of the pomegranate plant that makes up about 50% of the total fruit weight (Fawole and Opara 2016). Studies have shown that pomegranate peel has high antioxidant effect, coupled with

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its antimicrobial, hypoglycaemic, hypolipidemic, non-cytotoxic, hepatoprotective and anti-inflammatory properties (Belal et al. 2009; Rajput et al. 2011). Appreciable amounts of bioactive compounds have been isolated from the pomegranate peel. They include the hydrolysable tannins, such as ellagitannin, gallotannins, and the gallagyl esters like punicalagin, punocaliin and pedunculagin (Madrigal-Carballo et al. 2009). Pomegranate peel also contains substantial amounts of flavonoids, catechins, ellagic acid, flavonones, flavones, anthocyanidins, in addition to other polyphenolic constituents (Naveena et al. 2008).

The presence of these compounds endow pomegranate peel with reducing power and radical scavenging abilities (Rajani et al. 2011). Essential mineral elements, such as potassium, phosphorous, calcium, sodium, manganese, iron, nitrogen, are also reportedly found in pomegranate peel (Mirdehghan and Rahemi 2007). The hypoglycaemic and hypolipidaemic properties of pomegranate peel enable it to improve immune responses in broilers (Sharifian et al. 2019), quails (Abbas et al. 2017), fish (Badawi and Gomaa 2016), and cows (Safari et al. 2018). Pomegranate peel improves the oxidative stability of meat due to its antioxidant property (Descalzo et al. 2008). This improvement in oxidative stability is attributed to the scavenging effects of pomegranate peel on the active forms of reactive oxygen species (ROS), which has been implicated in the initiation and progressive phases of oxidation (Goliomytis et al. 2015). Vitamin E ( $\alpha$ -tocopherol acetate) is also an important synthetic antioxidant that coordinates several metabolic processes (Habibian et al. 2014). Vitamin E promotes growth, activates immune responses, increases carcass and cut yield, protects against free radicals, and invariably lipid peroxidation, and oxidative stress (Gao et al. 2010; Selim et al. 2013b; Habibian et al. 2014).

There are a number of inconsistencies in the literature on whether the powder, extract and pomace of pomegranate peel (PP) influence the growth performance of broiler birds and other livestock species. Whilst some reports have shown that PP (extract and powder) improved body weight, feed intake, feed efficiency, carcass and organ parameters in broilers (Al-Shammari et al. 2019; Kishawy et al. 2019; Sharifian et al. 2019; Abdel-Baset et al. 2020), and in quails (Abbas et al. 2017), there are reports that PP supplementation had a depressing effect on the performance parameters of broiler birds (Saleh et al. 2018). However, the findings from some studies have also shown that PP supplementation did not influence

broilers' growth performance, carcass and organ indices (Rajani et al. 2011; Sarica and Urkmez 2016; Sharifian et al. 2019; Abdel-Baset et al. 2020). Similarly, there are reports that PP supplementation decreases serum total cholesterol, triglycerides, alanine transaminase, alanine amino transferase levels in broilers, (Kishawy et al. 2019; Sharifian et al. 2019; Abdel-Baset et al. 2020) and quails (Abbas et al. 2017). The reports of Al-Shammari et al. (2019) showed that dietary inclusion of 1% pomegranate peel and 0.5%  $H_2O_2$  additive increased the plasma catalase activity in broiler birds.

Based on the aforementioned bioactive constituents of pomegranate peel, particularly, its antioxidant potentials the present study was carried out. More so, to the best of our knowledge, there is no existing literature on the dietary effects of pomegranate peel powder on the growth performance, nutrient digestibility, carcass and organ parameters, serum and some meat antioxidant biomarkers on Cobb 500 birds reared in South Africa. This is due to the fact that a number of researchers in South Africa exploring the potentials of pomegranate peel concentrate more on *in vitro* assays or other forms of assays that do not involve animal feeding. Moreover, much emphasis has been placed on the extract of pomegranate peel as opposed to the powder. To this end, the use of pomegranate peel powder in broiler nutrition is not yet exhaustive. Similarly, the South African grown 'Wonderful' pomegranate variety used in this study, has not been previously utilised in broiler nutrition.

In previous studies, the powder of pomegranate pomace was supplemented in broiler diets at 1, 2, and 3 g/kg (Saleh et al. 2018), whereas pomegranate peel powder was included in the diets of the broiler birds at 2, 3 and 4 g/kg (Abdel-Baset et al. 2020). Nonetheless, authors are not aware of existing literatures in which pomegranate peel powder was supplemented at higher inclusion levels (up to 6 and 8 g/kg) in the diets of broiler birds. Therefore, the objectives of this study were to determine the effect of pomegranate (South African grown 'Wonderful' variety) peel powder meal inclusion on the growth performance, digestibility, carcass and organ weight weights, serum and some meat antioxidant enzyme parameters of Cobb 500 broiler birds. It is hypothesised that varying dietary levels of pomegranate peel powder meal has effect on the growth performance, nutrient digestibility, carcass and organ parameters, serum and some meat antioxidant enzyme biomarkers of Cobb-500 birds.

## Materials and methods

### Ethical statement

Ethical approval for the study was sought and obtained from the Animal Research Ethics Committee of the University of Fort Hare, Alice (Ethical clearance number: MUC061SAKU01). Permission to conduct research was also obtained in terms of Section 20 of the Animal Diseases Act, 1984 from the Department of Agriculture, Forestry and Fisheries (DAFF), South Africa with reference number: 12/11/1/4.

### Study location and ingredients sources

The 35-day experimental feeding trial was carried out at the Poultry section of the Fort Cox College of Agriculture and Forestry Training institute located at Middledrift, Eastern Cape, South Africa on the following coordinates 32.46°S, 27.02°E. Fresh pomegranate (*Punica granatum*) peels were supplied by the Post-harvest research centre, Stellenbosch University. The vitamin E ( $\alpha$ -tocopherol acetate) used was procured from Merck (Pty) Ltd Modderfontein, South Africa. All other feed ingredients were procured from Monti Feeds (East London, South Africa).

### Peel collection and preparation

Fresh pomegranate peels ('Wonderful' variety) were obtained from the Post-harvest research centre of Stellenbosch University, and dried as prescribed by Mphahlele et al. (2016) with slight modifications; the peels were put in clean trays that were already weighed and put into the oven (Model No. 072160, Prolab instrument, Sep Sci., South Africa) at 60 °C. Changes in weight were recorded using a digital weighing balance (ML3002.E, Mettler Toledo, Switzerland) at an hourly interval during drying. The

moisture content of peels was determined by drying peel until it reached equilibrium, i.e. when no more changes in weight was recorded. Usually, a moisture content of 8% (weight basis) is reached after 22 hours. The resulting oven-dried peels were ground into powder using a milling machine to pass through a 0.15 mm sieve, stored at –20 °C and used for proximate and mineral analysis (Table 1) and feeding trial.

### Preparation of pomegranate peel extracts

About 2.5 g of dried pomegranate peel powder (PPP) was extracted using 80 mL ethanol solvent under shaking for 48 hours. The crude extract was filtered under pressure using a Buchner funnel and Whatman No. 1 filter paper. The filtrate was then concentrated under vacuum at 30 °C using a high capacity rotary evaporator (Strike 202 Steroglass, Italy). A lyophilizer (Vir Tis benchtop K, Vir Tis Co, Gardiner, NY) was used to dry the ethanol-free extract, after which the dried samples were kept at –70 °C until needed for analysis.

### Determination of phytochemical and antioxidant contents of PPP

Total polyphenol of the extract of PPP was determined using the Folin Ciocalteu's phenol reagent methods described by Singleton et al. (1998). The total phenol content of the extract was expressed as mg/g equivalent of gallic acid (mg/g GAE). Total antioxidant capacity of the extract was determined using the oxygen Radical Absorbance Capacity (ORAC) assay based on the fluourometric method prescribed by Ou et al. (2001). The Trolox Equivalent Antioxidant Capacity (TEAC) assay was done to determine the ABTS [(2, 2-azinobis (3ethylbenzothiazoline-6 sulphonic acid))] scavenging ability of the extract (Re et al. 1999). The method described by Benzie and Strain (1996) was used to determine the ferric reducing antioxidant power (FRAP) assay of the extract (Table 2).

**Table 1.** Proximate and mineral composition of pomegranate peel powder.

Parameter	Quantity
Crude protein (% as fed basis)	2.17
Moisture (%)	6.67
Ash (%)	4.06
Ether extract (%)	6.54
Acid detergent fibre (%)	26.90
Neutral detergent fibre (%)	34.50
Calcium (%)	1.05
Phosphorus (%)	1.24
Potassium (%)	1.82
Magnesium (%)	0.55
Sodium (%)	0.31
Copper (mg/kg)	37.00
Iron (mg/kg)	279.00
Zinc (mg/kg)	15.10
Manganese (mg/kg)	15.70

**Table 2.** Phytochemical and antioxidant contents of the extract of PP powder.

Parameter	Concentration
ORAC ( $\mu$ mol TE/g)	1006.29
FRAP ( $\mu$ mol AAE/g)	696.51
ABTS <sup>+</sup> ( $\mu$ mol TE/g)	507.93
Polyphenols (mg GAE/g)	143.98
Flavonols (mg QE/g)	16.75
Flavanols (mg CE/g)	N.D.

TE: trolox equivalents; AAE: ascorbic acid equivalents; GAE: gallic acid equivalents; QE: quercetin equivalents; CE: catechin equivalents; N.D: none detected; PP: pomegranate peel.

**Table 3.** Ingredients and nutrient composition of basal diet.

Ingredients	Starter (0–21 days)	Grower-finisher (22–35 days)
Maize	48.84	58.00
Soybean full fat	28.50	36.78
Soybean meal (CP 44.0%)	13.25	–
Fishmeal 65	4.00	–
L-Lysine Hcl	0.15	0.13
DL-Methionine	0.40	0.32
L-Threonine	0.16	0.05
Vit + min premix <sup>a</sup>	0.15	0.15
Limestone	1.46	1.40
Salt	0.20	0.25
Monocalcium phosphate	1.23	1.32
Sodium bicarbonate	0.16	0.10
Sunflower oil	1.50	0.15
Calculated composition, %		
ME, MJ/kg	13.18	13.81
Crude protein	24.07	19.38
Crude fibre	4.56	3.34
Ether extract	5.54	6.86
Calcium	1.03	1.01
Available phosphorous	0.44	0.37
Lysine	1.44	1.06
Threonine	0.89	0.70
Tryptophan	0.28	0.21
Analysed composition, %		
Crude protein	23.24	20.05
Ash	5.34	5.16
Ether extract	8.89	8.70
Acid detergent fibre (ADF)	4.63	4.86
Neutral detergent fibre (NDF)	14.44	20.09
Calcium, %	1.41	1.36
Phosphorus, %	0.78	1.23

<sup>a</sup>Vitamin + mineral premix provided (per kg of feed): 8160 U vit A, 1700 U vitamin D3, 30.6 U vitamin E, 2.7 mg vitamin K3, 205 mg vitamin B1, 2.03 mg vitamin B2, 27.2 mg niacin, 10.2 mg calcium pantothenate, 2.02 mg vitamin B12, 4.1 mg vitamin B6, 1.7 mg folic acid, 0.068 mg biotin, 120 mg ronozyme P500, 350 mg choline, 0.08 mg I, 0.34 mg Co, 0.2 mg Se, 70 mg Mn, 70 mg Zn, 6 mg C and 50 mg Fe.

### Experimental diets

The feeding strategy consisted of experimental starter (0–21 days), and grower-finisher (22–35 days) diets (Table 3) which were formulated to meet the dietary nutrient requirements of the birds (National Research Council 2008). The experimental diets were designated as: T<sub>1</sub> – control diet with 0% additives (negative control; NEGCON); T<sub>2</sub> – control diet supplemented with  $\alpha$ -tocopherol acetate at 200 g per ton (positive control; POSOCON); T<sub>3</sub> – control diet supplemented with 2 g/kg pomegranate peel powder meal (PPPM) (PPPM<sub>2</sub>); T<sub>4</sub> – control diet supplemented with 4 g/kg PPPM (PPPM<sub>4</sub>); T<sub>5</sub> – control diet supplemented with 6 g/kg PPPM (PPPM<sub>6</sub>); T<sub>6</sub> – control diet supplemented with 8 g/kg PPPM (PPPM<sub>8</sub>). The proximate contents of the diets (Table 3) were determined based on the methods described by the Association of Analytical Chemists (AOAC 2000). The methods described by van Soest et al. (1991) were used to determine the concentrations of acid detergent fibre (ADF) and neutral detergent fibre (NDF). Mineral composition of the diets

was determined following the guidelines of AgriLasa (1998).

### Experimental birds and management

A total of 432 mixed-sex Cobb 500 broiler chicks were used in the 35-day experimental trial. The broiler chicks were housed in a temperature-controlled house that contained wood shavings of 10 cm depth as litter material. Seventy-two birds were randomly assigned to one of six experimental diets. Each experimental diet was replicated in four experimental pens with 18 birds each in a completely randomised design (CRD). The temperature of the broiler house at the start of the feeding trial was set at 35 °C, and thereafter, reduced gradually by 2–3 °C weekly, until it reached 22 °C in the 5th week. A 24-hour lighting regimen per day was provided for the first 72 hours to stimulate feeding and drinking in the young chicks. This was reduced to 23 hours per day by the end of the first week (day 7) of life. After that, a step down lighting program was followed until slaughter. Artificial bulbs were used as the source of light. The birds were given Gumboro disease vaccine at days 7 and 14 of the feeding trial, while, New Castle disease vaccine was administered on 21 and 28 days of age. Dietary treatments and clean water was supplied to the birds *ad libitum* during the five weeks of the feeding trial.

### Growth performance traits

At the beginning of the feeding trial, birds in each pen were weighed, and subsequently on weekly basis using. Then, average daily body weight gain (g) was calculated by subtracting initial body weight (g) from final body weight (g) over the 35-day period. Average daily feed intake (g/bird/day) was calculated as the difference between quantity of feed offered and the quantity of feed refused, divided by the number of birds per replicate. Feed conversion ratio (FCR) was calculated by dividing feed intake per bird (g) by the weight gain per bird (g). Protein intake (PI) was calculated by expressing the amount of feed consumed by the birds as a percentage of the protein in diets. Protein efficiency was calculated as the weight gain (g) divided by protein consumed (g) over the period of the trial to determine the amount of protein utilised by the birds.



### Digestibility trial

On the 28th day of the feeding trial, one bird per replicate was randomly selected and transferred to clean and disinfected metabolic cages for a digestibility trial. Each metabolic cage was equipped with a small feeding and drinking trough, and had adequate openings for faecal matter collection. Clean stainless trays were placed underneath the cages for ease of faecal collection. Before the commencement of faecal collection, the birds were given an adjustment period of 3 days. Faecal collection was done on a daily basis per treatment for 4 days, from day 31 to day 35, immediately after the adjustment period. The faecal matter obtained per day in each collection tray was carefully screened for spilt feed, air dried at room temperature, ground finely and then used for the proximate determination of crude fibre, crude protein, ether extract and dry matter based the methods of AOAC (2006). The concentrations of acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined using the techniques described by van Soest et al. (1991). Digestibility was then calculated as:

$$\text{Nutrient Digestibility (\%)} = \frac{(\text{Nutrient in Feed} - \text{Nutrient in Excreta})}{(\text{Nutrient in Feed})} \times 100$$

### Serum biochemical indices

On the 35th day, 4 mL blood was drawn from one bird per replicate using sterile needles. The blood was collected into properly labelled non-heparinised tubes. After that, the serum was isolated and stored at  $-20^{\circ}\text{C}$  for biochemical analysis. The concentrations of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, aspartate aminotransferase, total protein, and albumin were determined using an Auto analyser (Hitachi-704, Boehringer Mannheim GmbH, Mannheim, Germany).

### Sampling for carcase and portion yield analysis

On the 35th day of the feeding trial, one bird per replicate was randomly selected and fasted for six hours. A record of the individual weight of each bird was taken using a weighing scale to ascertain live weight at slaughter (LWS). Birds were then stunned at 70 V and humanely slaughtered by cervical dislocation, followed by exsanguination. Immediately after slaughter, the birds were scalded, manually defeathered and eviscerated. After that, the carcasses were washed and

allowed to drip for 5 min, and then an incision was made around the vent region where the gastrointestinal tract and other organs were removed. The weights of individual carcase cut, visceral organs (gizzard, liver, spleen, heart, pancreas etc.) and the length of the small and large intestine were recorded. Dressing percentage, portion yields, and relative organ weights were then calculated accordingly.

### Sampling for catalase enzyme activity

Pectoralis major samples were taken from the birds on the 35th day, stored at  $4^{\circ}\text{C}$  and used for the determination of catalase (CAT) enzyme activity. Before analysis, sub-samples of breast meat samples from broilers were deproteinised with 0.5 M perchloric acid (1:1, v/v) and centrifuged at  $10,000 \times g$  at  $4^{\circ}\text{C}$ . Supernatant collected was a protein-free fraction stored at  $-80^{\circ}\text{C}$  until required for analysis (Robles-Sanchez et al. 2011). CAT activity in the breast meat samples was determined based on the methods of Aebi (1984) which involves measuring the rate of  $\text{H}_2\text{O}_2$  decomposition at 232 nm and also expressed as U/mg protein.

### Statistical analysis

The PROC MIXED procedure of SAS (2010) for repeated measures was used to test for significance of diets on average weekly feed intake (WFI), average weekly weight gain (WWG) and average weekly feed conversion efficiency (WFCE). Before analysis, all reported weekly growth parameters were tested for normality using the 'Normal' option in the Proc Univariate statement. The following statistical linear model was used:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ijk}$$

where  $Y_{ijk}$  = dependent variable;  $\mu$  = overall mean common to all observations;  $D_i$  = effect of dietary treatments;  $W_j$  = effect of week;  $(D \times W)_{ij}$  = effect of interaction between diets and week;  $E_{ijk}$  = random error associated with observation  $ijk$ , assumed to be normally and independently distributed.

The weekly growth measurements (WFI, WWG and WFCE) were used as covariates.

Data generated from the starter, finisher, and overall phases of the study on growth indices, visceral organ weights, and apparent nutrient digestibility was analysed using the GLM Procedures of SAS (2010), with the statistical model:

$$Y_{ij} = \mu + D_i + E_{ij}$$

where  $Y_{ij}$  = Observed value of a dependent variable;

**Table 4.** Average weekly feed intake, weight gain, and feed conversion ratio of broiler birds fed pomegranate peel powder.

Treatments	NEGCON	POSCON	PPPM <sub>2</sub>	PPPM <sub>4</sub>	PPPM <sub>6</sub>	PPPM <sub>8</sub>	SEM	<i>p</i> Value
Feed intake, g bird <sup>-1</sup>								
Week 1	101.02	103.70	92.49	106.41	113.15	108.88	1.43	.15
Week 2	351.93	347.10	356.29	362.19	346.00	353.02	2.74	.19
Week 3	607.80 <sup>b</sup>	582.98 <sup>c</sup>	619.68 <sup>a</sup>	607.00 <sup>b</sup>	605.84 <sup>b</sup>	609.84 <sup>b</sup>	8.79	.02
Week 4	948.06	942.25	915.28	933.90	899.64	904.24	6.67	.33
Week 5	1080.40	1084.20	1046.80	1046.10	1053.10	1026.00	8.78	.64
Body weight, g bird <sup>-1</sup>								
Week 1	180.00	171.80	171.62	185.54	171.90	171.29	1.35	.25
Week 2	445.56	426.10	421.56	452.53	436.78	419.56	4.85	.67
Week 3	890.79	861.71	833.00	873.50	912.26	840.84	10.29	.15
Week 4	1443.37	1366.69	1405.10	1360.30	1432.60	1345.10	18.23	.41
Week 5	2097.10 <sup>ab</sup>	2007.60 <sup>b</sup>	2179.60 <sup>a</sup>	2138.20 <sup>a</sup>	1993.00 <sup>b</sup>	2068.80 <sup>ab</sup>	14.77	.01
Feed conversion ratio								
Week 1	0.56	0.60	0.54	0.57	0.66	0.64	0.67	.54
Week 2	0.79	0.81	0.85	0.80	0.79	0.84	0.82	.63
Week 3	0.68 <sup>b</sup>	0.68 <sup>b</sup>	0.74 <sup>a</sup>	0.69 <sup>b</sup>	0.66 <sup>b</sup>	0.73 <sup>a</sup>	0.70	.01
Week 4	0.67	0.69	0.65	0.69	0.63	0.67	0.66	.35
Week 5	0.52	0.54	0.48	0.49	0.53	0.50	0.51	.12

<sup>a,b,c</sup>Means within columns with different superscripts differ significantly ( $p < .05$ ).

NEGCON: negative control diet (0% additives); POSCON: positive control diet with 200 g  $\alpha$ -tocopherol acetate per ton; PPPM: pomegranate peel powder meal at different inclusion levels of 2, 4, 6 and 8 g/kg; FCR: feed conversion ratio; SEM: standard error of mean.

$\mu$  = Overall mean;  $D_i$  = effect of dietary treatments;  $E_{ij}$  = Residual error associated with observation  $ij$ .

All statistical tests were declared significant at  $p < .05$ . Means separation was done using the Duncan's New Multiple Range Test of SAS (2010). Data generated were presented as least square means with standard error of means.

## Results

### Growth performance

#### Weekly growth traits

The results for feed intake (FI), feed conversion ratio (FCR) and body weight (BW) of broilers fed varying dietary levels of pomegranate peel powder meal (PPPM) are shown in Table 4. There were significant ( $p < .05$ ) interaction between diet and week for FI and FCR at week 3. At week 3, FI was highest for birds fed PPPM<sub>2</sub> (2 g/kg PPPM), while birds fed the positive control (POSCON) had the lowest FI. Birds fed the negative control (NEGCON), PPPM<sub>4</sub> (4 g/kg PPPM), PPPM<sub>6</sub> (6 g/kg PPPM) and PPPM<sub>8</sub> (8 g/kg PPPM) had similar FI values. The weekly FCR showed that at week 3, birds fed POSCON consumed less feed to produce less kilogram of tissue. However the FCR value of POSCON birds was similar ( $p > .05$ ) to the FCR recorded for birds fed the NEGCON, PPPM<sub>4</sub> and PPPM<sub>6</sub> diets with slightly higher FI. This showed that birds fed the controls (NEGCON and POSCON) and the PPPM<sub>4</sub> and PPPM<sub>6</sub> diets had improved FCR at week 3.

### Growth parameters for the different experimental phases

As shown in Table 5, dietary supplementation of pomegranate peel powder meal (PPPM) had significant ( $p < .05$ ) influence on the growth traits of the broiler birds. At the starter phase (0–21 days), daily protein intake (DPI) was significantly improved in birds fed PPPM<sub>2</sub>, PPPM<sub>4</sub>, NEGCON and POSCON diets compared with those fed the PPPM<sub>8</sub> diet. Protein efficiency ratio (PER) was highest in the PPPM<sub>2</sub> birds compared with birds fed the POSCON and other dietary levels of PPPM. Birds fed NEGCON and PPPM<sub>2</sub> diets had similar PER values. At the finisher (22–35 days) phase, PPPM<sub>2</sub> and PPPM<sub>4</sub>-treated birds had highest average final body weight (AFBW) compared with birds fed POSCON and PPPM<sub>6</sub> diets. However, AFBW was similar ( $p > .05$ ) among birds fed PPPM<sub>8</sub>, NEGCON and the PPPM<sub>2</sub> and PPPM<sub>4</sub> groups. Although, PER and FCR were significantly improved in the PPPM<sub>2</sub> birds compared with POSCON and PPPM<sub>6</sub>, these values did not differ from that of those recorded for birds fed other treatment diets. The NEGCON and POSCON birds had higher FI compared with birds fed the PPPM<sub>8</sub> diet. Similar ( $p > .05$ ) FI values were recorded for the control birds and the PPPM<sub>2</sub>, PPPM<sub>4</sub> and PPPM<sub>6</sub> dietary groups. At the overall phase, average daily weight gain (ADWG) was higher in the PPPM<sub>2</sub> and PPPM<sub>4</sub> birds compared to those of the POSCON and PPPM<sub>6</sub>; however these values were similar to the ADWG of birds fed other dietary treatments (Table 5).



**Table 5.** Effect of pomegranate peel powder meal on performance of broilers.

	Experimental diets							
Parameters	NEGCON	POSCON	PPPM <sub>2</sub>	PPPM <sub>4</sub>	PPPM <sub>6</sub>	PPPM <sub>8</sub>	SEM	<i>p</i> Value
0–21 days (Starter phase)								
Total BW, g	890.79	861.71	833.00	873.50	912.26	840.84	10.75	.26
Feed intake g,	1069.04	1033.78	1068.47	1075.59	1064.47	1071.75	8.77	.82
FCR, g/g	1.20	1.21	1.28	1.23	1.18	1.27	0.02	.41
Daily PI g	11.83 <sup>a</sup>	11.73 <sup>a</sup>	12.03 <sup>a</sup>	11.81 <sup>a</sup>	11.44 <sup>ab</sup>	10.87 <sup>b</sup>	0.11	.00
PER	3.59 <sup>ab</sup>	3.49 <sup>b</sup>	3.83 <sup>a</sup>	3.49 <sup>b</sup>	3.47 <sup>b</sup>	3.39 <sup>b</sup>	0.05	.04
22–35 days (Finisher phase)								
AFBW, g	2097.10 <sup>ab</sup>	2007.60 <sup>b</sup>	2179.60 <sup>a</sup>	2138.20 <sup>a</sup>	1993.00 <sup>b</sup>	2068.80 <sup>ab</sup>	17.30	.01
Total BW, g	1206.30 <sup>ab</sup>	1145.90 <sup>b</sup>	1346.60 <sup>a</sup>	1264.80 <sup>ab</sup>	1080.70 <sup>b</sup>	1227.90 <sup>ab</sup>	27.21	.06
Feed intake, g	2028.5 <sup>a</sup>	2026.40 <sup>a</sup>	1962.1 <sup>ab</sup>	1980.00 <sup>ab</sup>	1952.8 <sup>ab</sup>	1930.3 <sup>b</sup>	12.68	.12
FCR, g/g	1.68 <sup>ab</sup>	1.77 <sup>a</sup>	1.46 <sup>b</sup>	1.57 <sup>ab</sup>	1.81 <sup>a</sup>	1.57 <sup>ab</sup>	0.02	.01
DPI, g	29.05	29.17	28.45	28.52	28.01	27.92	0.18	.21
PER	3.30 <sup>ab</sup>	3.12 <sup>b</sup>	3.71 <sup>a</sup>	3.49 <sup>ab</sup>	3.09 <sup>b</sup>	3.47 <sup>ab</sup>	0.05	.01
0–35 days (Overall phase)								
Initial BW, g	42.20	41.08	40.04	42.60	42.76	41.43	0.30	.07
AFBW, g	2097.10 <sup>ab</sup>	2007.60 <sup>b</sup>	2179.60 <sup>a</sup>	2138.20 <sup>a</sup>	1993.00 <sup>b</sup>	2068.80 <sup>ab</sup>	17.31	.01
Body WG, g	2054.93	1966.54	2139.59	2095.66	1950.24	2027.42	28.28	.37
ADWG, g	59.92 <sup>ab</sup>	57.36 <sup>b</sup>	62.28 <sup>a</sup>	61.09 <sup>a</sup>	56.94 <sup>b</sup>	59.11 <sup>ab</sup>	0.49	.01
Total FI, g	3097.52	3060.21	3033.56	3055.60	3017.76	3002.01	14.25	.47
ADFI, g	85.76	86.23	87.30	87.44	87.44	88.49	0.05	.47
FCR, g/g	1.50	1.56	1.42	1.46	1.55	1.48	0.02	.32
DPI, g	17.74	17.62	17.58	17.61	17.32	17.33	0.08	.63
PER	3.38	3.25	3.55	3.47	3.29	3.41	0.05	.46

<sup>a,b,c</sup>Means within columns with different superscripts differ significantly ( $p < .05$ ).

NEGCON: negative control diet (0% additives); POSCON: positive control diet with 200 g  $\alpha$ -tocopherol acetate per ton; PPPM<sub>2</sub>: 2 g/kg PPPM; PPPM<sub>4</sub>: 4 g/kg PPPM; PPPM<sub>6</sub>: 6 g/kg PPPM; PPPM<sub>8</sub>: 8 g/kg PPPM; SEM: standard error of mean; BW: body weight; PI: protein intake; PER: protein efficiency ratio; WG: weight gain; ADWG: average daily weight gain; ADFI: average daily feed intake; FI: feed intake; FCR: feed conversion ratio; DPI: daily protein intake.

**Table 6.** Apparent digestibility of broilers fed pomegranate powder peel meal.

Parameters %	Experimental diets						SEM	p Value
	NEGCON	POSCON	PPPM <sub>2</sub>	PPPM <sub>4</sub>	PPPM <sub>6</sub>	PPPM <sub>8</sub>		
Dry Matter	66.86 <sup>ab</sup>	58.00 <sup>c</sup>	70.13 <sup>a</sup>	69.32 <sup>a</sup>	63.74 <sup>b</sup>	57.68 <sup>c</sup>	1.27	.00
Ether extract	68.85 <sup>b</sup>	65.17 <sup>b</sup>	73.32 <sup>a</sup>	74.19 <sup>a</sup>	65.29 <sup>b</sup>	60.07 <sup>c</sup>	1.15	.00
Ash	64.78 <sup>a</sup>	55.42 <sup>b</sup>	63.88 <sup>a</sup>	62.46 <sup>a</sup>	56.66 <sup>b</sup>	54.27 <sup>b</sup>	1.12	.00
Crude protein	78.55	78.31	75.81	76.04	77.75	78.55	0.73	.85
ADF	62.38 <sup>b</sup>	61.37 <sup>bc</sup>	63.93 <sup>ab</sup>	67.99 <sup>a</sup>	63.20 <sup>ab</sup>	57.18 <sup>c</sup>	0.95	.02
NDF	65.22 <sup>bc</sup>	61.94 <sup>c</sup>	68.11 <sup>ab</sup>	68.94 <sup>a</sup>	58.51 <sup>d</sup>	56.15 <sup>d</sup>	1.21	.00
NFE	64.11 <sup>ab</sup>	60.02 <sup>bc</sup>	64.36 <sup>ab</sup>	64.65 <sup>a</sup>	57.90 <sup>cd</sup>	54.25 <sup>c</sup>	1.05	.00

<sup>a,b,c,d</sup>Means within columns with different superscripts differ significantly ( $p < .05$ ).

NEGCON: negative control diet (0% additives); POSCON: positive control diet with 200 g  $\alpha$ -tocopherol acetate per ton; PPPM<sub>2</sub>: 2 g/kg PPPM; PPPM<sub>4</sub>: 4 g/kg PPPM; PPPM<sub>6</sub>: 6 g/kg PPPM; PPPM<sub>8</sub>: 8 g/kg PPPM; ADF: acid detergent fibre; NDF: neutral detergent fibre; NFE: nitrogen-free extract; SEM: standard error of mean.

### Apparent nutrient digestibility

The results presented in Table 6 showed that dry matter (DM) digestibility was highest ( $p < .05$ ) in the PPPM<sub>2</sub> and PPPM<sub>4</sub> birds compared with the POSCON, PPPM<sub>6</sub> and PPPM<sub>8</sub> birds. Birds fed NEGCON had similar ( $p > .05$ ) DM digestibility with the PPPM<sub>2</sub>, PPPM<sub>4</sub> and PPPM<sub>6</sub> birds. Ash digestibility was significantly improved in the PPPM<sub>2</sub>, PPPM<sub>4</sub> and NEGCON birds compared with birds fed other treatment diets. Ether extract (EE) digestibility was improved in birds fed the PPPM<sub>2</sub> and PPPM<sub>4</sub> diets compared with those that received other dietary treatments. The digestibility of the acid detergent fibre (ADF) and neutral detergent fibre (NDF) was improved ( $p < .05$ ) in the PPPM<sub>4</sub>-treated birds compared with the NEGCON,

POSCON, PPPM<sub>6</sub> and PPPM<sub>8</sub> birds. Birds fed the NEGCON, PPPM<sub>2</sub> and PPPM<sub>4</sub> diets had the highest ( $p < .05$ ) nitrogen-free extract (NFE) digestibility compared with those in the PPPM<sub>6</sub> and PPPM<sub>8</sub> dietary groups.

### Carcase traits and portion yield

The results (Table 7) on carcase traits and portion yield of broilers fed diets supplemented with PPPM did not follow a linear trend. The weight of drumstick was similar ( $p > .05$ ) across treatments. Birds fed PPPM<sub>8</sub> diet had the highest breast weight compared with the POSCON and PPPM<sub>2</sub> diets; this however was not different ( $p > .05$ ) from the breast weights of birds on other

**Table 7.** Carcase and portion yield of broilers fed pomegranate powder peel meal.

Parameter	Experimental diets						SEM	p Value
	NEGCON	POSCON	PPPM <sub>2</sub>	PPPM <sub>4</sub>	PPPM <sub>6</sub>	PPPM <sub>8</sub>		
LW, g	2082.00 <sup>ab</sup>	2007.60 <sup>b</sup>	2179.60 <sup>a</sup>	2138.20 <sup>a</sup>	1993.00 <sup>b</sup>	2068.80 <sup>ab</sup>	27.36	.02
HCW, g	1513.00	1438.50	1612.50	1547.1	1456.6	1528.60	23.28	.30
Dressing %	73.04	71.96	74.17	72.62	73.12	74.46	1.44	.99
Drumstick, g	93.25 <sup>ab</sup>	95.25 <sup>ab</sup>	100.63 <sup>a</sup>	98.25 <sup>a</sup>	101.88 <sup>a</sup>	95.63 <sup>ab</sup>	2.29	.05
Breast, g	424.13 <sup>ab</sup>	411.13 <sup>b</sup>	410.00 <sup>b</sup>	434.13 <sup>ab</sup>	434.13 <sup>ab</sup>	461.75 <sup>a</sup>	14.40	.02
Thigh, g	124.63 <sup>a</sup>	106.38 <sup>b</sup>	121.25 <sup>ab</sup>	127.25 <sup>a</sup>	107.63 <sup>b</sup>	117.75 <sup>ab</sup>	2.45	.04
Wing, g	78.38 <sup>ab</sup>	76.88 <sup>b</sup>	86.25 <sup>a</sup>	80.75 <sup>ab</sup>	74.25 <sup>b</sup>	78.88 <sup>ab</sup>	1.54	.05
Shank, g	38.25	36.25	36.63	38.88	38.13	36.88	0.91	.97
Head, g	51.93	53.25	55.38	54.50	53.63	53.13	0.63	.75
Neck, g	107.79 <sup>a</sup>	77.88 <sup>b</sup>	84.50 <sup>b</sup>	80.00 <sup>b</sup>	82.13 <sup>b</sup>	82.88 <sup>b</sup>	3.09	.04
Back, g	209.88 <sup>a</sup>	198.50 <sup>ab</sup>	193.88 <sup>ab</sup>	213.13 <sup>a</sup>	179.75 <sup>b</sup>	211.13 <sup>a</sup>	6.45	.05
Yield (%)								
Breast	24.91	25.24	22.79	25.68	26.15	26.56	0.85	.87
Thigh	7.30	6.53	6.69	7.29	6.49	6.84	0.15	.43
Wing	4.57	4.72	4.75	4.63	4.48	4.58	0.08	.95
Drumstick	5.47	5.83	5.54	5.64	6.16	5.55	0.15	.82
Back	12.30 <sup>a</sup>	12.19 <sup>a</sup>	10.75 <sup>b</sup>	12.21 <sup>a</sup>	10.83 <sup>b</sup>	12.22 <sup>a</sup>	0.39	.05
Head	3.05	3.27	3.06	3.12	3.23	3.09	0.49	.74
Neck	6.38 <sup>a</sup>	4.76 <sup>b</sup>	4.65 <sup>b</sup>	4.59 <sup>b</sup>	4.96 <sup>b</sup>	4.80 <sup>b</sup>	0.20	.05
Shank	2.22	2.22	2.13	2.22	2.29	2.14	0.44	.92

<sup>a,b,c</sup>Means within columns with different superscripts differ significantly ( $p < .05$ ).

NEGCON: negative control diet (0% additives); POSCON: positive control diet with 200 g  $\alpha$ -tocopherol acetate per ton; PPPM<sub>2</sub>: 2 g/kg PPPM; PPPM<sub>4</sub>: 4 g/kg PPPM; PPPM<sub>6</sub>: 6 g/kg PPPM; PPPM<sub>8</sub>: 8 g/kg PPPM; LW: live weight; HCW: hot carcase weight; SEM: standard error of mean.

treatment diets. The highest ( $p < .05$ ) thigh weights were recorded for the NEGCON and PPPM<sub>4</sub> birds compared with the POSCON and PPPM<sub>6</sub> birds. Thigh weights were similar ( $p > .05$ ) among the PPPM<sub>2</sub>, PPPM<sub>8</sub>, PPPM<sub>4</sub> and NEGCON birds. Although, wing weight was highest for the PPPM<sub>2</sub> compared with POSCON and PPPM<sub>6</sub>, this did not differ ( $p > .05$ ) from the wing weights recorded for birds fed other dietary treatments. The NEGCON birds had the highest neck weight compared with birds fed other treatment diets, whereas, birds fed NEGCON, PPPM<sub>4</sub> and PPPM<sub>8</sub> diet had the highest weight for back compared with the PPPM<sub>6</sub> group. In terms of portion yield, birds fed NEGCON, POSCON, PPPM<sub>4</sub> and PPPM<sub>8</sub> diets had the highest yield for back, compared with the PPPM<sub>2</sub> and PPPM<sub>6</sub> birds, whereas neck portion yield was highest for the NEGCON birds.

### Organ weights

The results on absolute and relative organ weights presented in Table 8 did not follow a linear trend. The NEGCON, POSCON and PPPM<sub>2</sub> birds diet had heavier ( $p < .05$ ) proventriculi compared with birds in the PPPM<sub>6</sub> group. Although, birds fed POSCON diet had the highest ( $p < .05$ ) heart weight compared with those fed the PPPM<sub>6</sub> diet, their heart weight was similar ( $p > .05$ ) to those recorded for birds fed other treatment diets. Liver weight was highest in the NEGCON, POSCON and PPPM<sub>2</sub> birds compared with the PPPM<sub>6</sub> and PPPM<sub>8</sub> groups. The PPPM<sub>4</sub> birds had the highest absolute spleen and gizzard weights compared with

those fed the NEGCON diet. Similar spleen weights existed among the PPPM<sub>4</sub> and the POSCON birds, while gizzard weight was similar ( $p > .05$ ) among the POSCON, PPPM<sub>2</sub> and PPPM<sub>4</sub> birds. Small intestine was longest ( $p < .05$ ) in birds fed the PPPM<sub>2</sub> diet compared with those that received the 4 g/kg and 8 g/kg PPPM diets. Small intestine lengths did not however differ ( $p > .05$ ) among the NEGCON, POSCON, PPPM<sub>6</sub> and the PPPM<sub>2</sub> birds. Birds fed the PPPM and control diets did not differ ( $p > .05$ ) in large intestine weights. The highest ( $p < .05$ ) relative proventriculi weight was recorded for POSCON birds compared with the PPPM<sub>4</sub>, PPPM<sub>6</sub> and PPPM<sub>8</sub> birds. Birds fed the POSCON diet had the highest gizzard relative weight compared with the PPPM<sub>8</sub> birds; however, this weight was similar ( $p > .05$ ) to the relative gizzard weights recorded for birds fed other treatment diets. The relative liver weight did not differ ( $p > .05$ ) among the PPPM-treated birds and the control groups.

### Blood serum indices and breast meat enzyme activity

As shown in Table 8, the highest ( $p < .05$ ) total protein was recorded in NEGCON birds compared with the PPPM<sub>4</sub> and PPPM<sub>8</sub> birds. Total protein values were similar ( $p > .05$ ) among the NEGCON, PPPM<sub>2</sub>, PPPM<sub>6</sub> and POSCON birds. Albumin was highest in the POSCON and the PPPM fed birds compared with the NEGCON. The concentration of aspartate aminotransaminase (AST) was significantly ( $p < .05$ ) reduced in PPPM<sub>4</sub>-treated birds compared with birds fed other dietary

**Table 8.** Organ weights of broilers fed pomegranate peel powder peel meal.

Parameter	Experimental diets						SEM	p Value
	NEGCON	POSCON	PPPM <sub>2</sub>	PPPM <sub>4</sub>	PPPM <sub>6</sub>	PPPM <sub>8</sub>		
Heart, g	11.75 <sup>ab</sup>	13.25 <sup>a</sup>	9.38 <sup>ab</sup>	11.38 <sup>ab</sup>	8.38 <sup>b</sup>	12.13 <sup>ab</sup>	0.63	.02
Spleen, g	1.35 <sup>b</sup>	1.63 <sup>ab</sup>	1.38 <sup>b</sup>	2.00 <sup>a</sup>	1.00 <sup>c</sup>	1.13 <sup>c</sup>	0.15	.04
Liver, g	39.13 <sup>a</sup>	39.25 <sup>a</sup>	40.13 <sup>a</sup>	37.25 <sup>ab</sup>	32.00 <sup>b</sup>	33.00 <sup>b</sup>	1.11	.04
Proventriculus	10.25 <sup>ab</sup>	11.38 <sup>a</sup>	9.00 <sup>ab</sup>	7.25 <sup>bc</sup>	4.75 <sup>c</sup>	7.25 <sup>bc</sup>	0.62	.01
Gizzard, g	51.63 <sup>b</sup>	57.88 <sup>ab</sup>	55.88 <sup>ab</sup>	61.88 <sup>a</sup>	50.50 <sup>b</sup>	49.75 <sup>b</sup>	1.68	.02
SI weight, g	88.13	101.50	97.88	98.25	86.75	88.75	2.45	.36
SIL, cm	167.25 <sup>abc</sup>	188.50 <sup>ab</sup>	191.50 <sup>a</sup>	159.75 <sup>bc</sup>	172.25 <sup>abc</sup>	148.25 <sup>c</sup>	4.65	.03
LIL, cm	13.75 <sup>ab</sup>	13.50 <sup>ab</sup>	16.25 <sup>a</sup>	9.75 <sup>b</sup>	16.75 <sup>a</sup>	15.25 <sup>ab</sup>	0.83	.02
Organ wt. %								
Heart	0.57	0.67	0.43	0.54	0.42	0.60	0.34	.25
Liver	1.89 <sup>a</sup>	1.97 <sup>a</sup>	1.84 <sup>a</sup>	1.75 <sup>ab</sup>	1.60 <sup>ab</sup>	1.59 <sup>ab</sup>	0.53	.04
Proventriculus	0.49 <sup>ab</sup>	0.56 <sup>a</sup>	0.41 <sup>abc</sup>	0.34 <sup>bc</sup>	0.24 <sup>c</sup>	0.36 <sup>bc</sup>	0.61	.02
Spleen	0.07	0.08	0.06	0.09	0.05	0.06	0.01	.53
Gizzard	2.49 <sup>ab</sup>	2.89 <sup>a</sup>	2.57 <sup>ab</sup>	2.90 <sup>a</sup>	2.53 <sup>ab</sup>	2.42 <sup>b</sup>	0.88	.05

<sup>a,b,c</sup>Means within columns with different superscripts differ significantly ( $p < .05$ ).

NEGCON: negative control diet (0% additives); POSCON: positive control diet with 200 g  $\alpha$ -tocopherol acetate per ton; PPPM<sub>2</sub>: 2 g/kg PPPM; PPPM<sub>4</sub>: 4 g/kg PPPM; PPPM<sub>6</sub>: 6 g/kg PPPM; PPPM<sub>8</sub>: 8 g/kg PPPM; SI: small intestine; SIL: small intestine length; LIL: large intestine length; wt: Weight; SEM: standard error of mean.

treatments. There were no dietary treatment effects on the other serum-biochemical traits evaluated. Catalase activity was highest ( $p < .05$ ) in the breast meat of PPPM<sub>8</sub>-treated birds compared with meats from other groups.

## Discussion

The diet  $\times$  week interaction effect on FI and FCR showed that inclusion of tocopherol acetate (POSCON diet) promoted a better FI and FCR at week 3. However, at this week, the FCR of the POSCON birds was comparable to that of the NEGCON, PPPM<sub>4</sub> and PPPM<sub>6</sub> (Table 4). Vitamin E ( $\alpha$ -tocopherol acetate) is an essential antioxidant compound with growth enhancing properties and ability to protect biological systems against free radicals and the deteriorating impact of oxidative stress (Selim et al. 2013b; Habibian et al. 2014) amongst other positive effects. Plant-based feed additives such as pomegranate peel also has positive influence on intestinal bacteria that enable birds to absorb more nutrients, leading to overall improvement in their feed utilisation (Middha et al. 2013). Earlier work by Thema et al. (2019) showed that there were no diet  $\times$  week interaction effect on FI, BWG and FCR of broiler birds when they were fed different combinations of probiotics and other feed additives.

There was a significant improvement in the AFBW and ADWG of birds fed 2 g/kg and 4 g/kg dietary pomegranate peel powder meal (PPPM) supplementation, whereas, FCR was improved at 2 g/kg PPPM inclusion compared with the tocopherol supplementation (POSCON diet) (Table 5). It appears that the 2 g/kg and 4 g/kg supplemented birds had better capacity to compete with the NEGCON birds in terms of improved

AFBW, ADWG and FCR compared with those fed the POSCON. This improvement in the PPPM<sub>2</sub> and PPPM<sub>4</sub> birds may perhaps be due to the growth promoting benefits of pomegranate peel which has been linked to its antioxidant and antimicrobial properties. The antioxidant effect of pomegranate peel is due to its possession of proanthocyanidin. The presence of proanthocyanidin in pomegranate peel enables it to improve pancreatic and small intestinal digestive enzyme functions, and prevent the deleterious influence of free radicals on intestinal enterocytes; thus, leading to enhanced nutrient absorption and use (Tavarez et al. 2011; Middha et al. 2013; Reddy et al. 2014). The antibacterial and antimicrobial potential of pomegranate peel is associated with its tannin content. The tannins enables pomegranate peel to decrease the population of harmful gut microbes, inhibit pathogenic microbial metabolism and the activities of harmful microbial enzymes by preventing oxidative phosphorylation (Viuda-Martos et al. 2010). These effects lead to overall improvement in the availability and absorption of nutrients in the intestinal lumen, with resultant improvement in bird performance (Abdollahzadeh et al. 2011; Mamdouh et al. 2015).

The findings of the present study on AFBW and FCR disagree with the findings of Saleh et al. (2018) who reported that dietary supplementation of pomegranate pomace powder at levels of 1, 2 and 3 g/kg impaired body weight, feed intake and feed conversion efficiency of broiler birds. Our result on FCR agrees with the findings of Ahmadipour et al. (2018).

There was a significant improvement in the PER of birds fed 2 g/kg PPPM supplemented diets at the starter and finisher phases compared with the POSCON diet (Table 5). The better nutrient (protein)

**Table 9.** Serum and meat enzyme indices of broilers fed pomegranate peel PM.

Treatments	NEGCON	POSCON	PPPM <sub>2</sub>	PPPM <sub>4</sub>	PPPM <sub>6</sub>	PPPM <sub>8</sub>	SEM	<i>p</i> Value
Serum-biochemical indices								
TP, g/dL	31.25 <sup>a</sup>	28.50 <sup>ab</sup>	29.25 <sup>ab</sup>	26.25 <sup>b</sup>	28.75 <sup>ab</sup>	27.25 <sup>b</sup>	0.55	.05
Albumin, g/dL	7.54 <sup>c</sup>	7.93 <sup>a</sup>	7.90 <sup>a</sup>	7.76 <sup>b</sup>	7.91 <sup>a</sup>	8.25 <sup>a</sup>	0.03	.00
AST, U/L	289.25 <sup>a</sup>	236.75 <sup>b</sup>	294.33 <sup>a</sup>	206.00 <sup>c</sup>	272.25 <sup>a</sup>	299.50 <sup>a</sup>	13.92	.03
ALT, U/L	5.98	5.45	5.50	6.50	5.75	6.75	0.24	.59
TC, mmol/L	2.97	3.02	3.26	2.99	3.33	2.96	0.08	.75
HDL-C, mmol/L	2.16	2.32	2.46	2.38	2.54	2.19	0.06	.58
LDL-C, mmol/L	0.72	0.62	0.68	0.53	0.73	0.64	0.04	.66
TG, mmol/L	0.21	0.17	0.25	0.19	0.43	0.28	0.04	.34
Meat antioxidant enzyme activity								
CAT, U/mg protein	0.33 <sup>b</sup>	0.30 <sup>b</sup>	0.33 <sup>b</sup>	0.46 <sup>b</sup>	0.42 <sup>b</sup>	0.79 <sup>a</sup>	0.05	.01

<sup>a,b,c</sup>Means within columns with different superscripts differ significantly ( $p < .05$ ).

PM: peel meal; NEGCON: negative control diet (0% additives); POSCON: positive control diet with 200 g  $\alpha$ -tocopherol acetate per ton; PPPM<sub>2</sub>: 2 g/kg PPPM; PPPM<sub>4</sub>: 4 g/kg PPPM; PPPM<sub>6</sub>: 6 g/kg PPPM; PPPM<sub>8</sub>: 8 g/kg PPPM; AST: aspartate aminotransferase; ALT: alanine amino transaminase; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; TG: triglycerides; CAT: catalase; TC: Total cholesterol; SEM: standard error of mean.

utilisation efficiency recorded in the PPPM<sub>2</sub> birds may be the reason for their improved growth response compared to the POSCON group. Pomegranate peel improves digestive enzyme functions and increases the efficiency of nutrient utilisation due to the possession of bioactive compounds (Al-Zoreky 2009; Banerjee et al. 2013; Reddy et al. 2014). The positive effects attributed to pomegranate peel is linked to the content of phenols, flavonoids (quercentin, kaemferol), tannins (ellagitannins, gallotannins), anthocyanidins (cyanidins), gallagyl esters (punicalagin), catechins, amongst others (Abdollahzadeh et al. 2011; Kishawy et al. 2019). Our findings align with the study of Kishawy et al. (2019) who reported that inclusion of 0.05% pomegranate peel extract in combination with either soybean oil or linseed oil enhanced the PER of broiler birds.

There was improvement in ether extract digestibility noted among the PPPM<sub>2</sub> and PPPM<sub>4</sub> birds, and in the digestibility of ADF and NDF recorded in the PPPM<sub>4</sub> birds compared with the NEGCON and the POSCON birds (Table 6). The digestibility of DM and NFE was also highest in the PPPM<sub>4</sub> birds, whereas ash digestibility was highest in the NEGCON, PPPM<sub>2</sub> and PPPM<sub>4</sub> compared with the POSCON birds (Table 5). This improvement in nutrient digestibility points to the fact that as a natural herb, pomegranate peel modulates digestive secretions and enzymatic functions, ensures beneficial activities within the gastrointestinal tract, and encourages overall growth stimulation in broilers. These assertions have been affirmed by earlier reports (Banerjee et al. 2013; Murugesan et al. 2015). Natural feed additives are also known to improve growth performance of broiler birds by regulating pre-cacal nutrient digestion, through a decrease in bacteria colony counts and associated fermentation products, and reduction in gut related lymphatic system actions (Amad et al.

2011). Murugesan et al. (2015) reported that the dietary inclusion of a phyto-genic-feed additive blend in the diets of broiler birds significantly increased ether extract digestibility in the birds.

The results in Table 7 showed that thigh weight was highest in the PPPM<sub>4</sub> birds, whereas PPPM<sub>8</sub> birds had the highest breast weight in comparison with the POSCON group. These results showed that at 4 g/kg and 8 g/kg supplementation of pomegranate peel was able to increase the proportions of prime parts such as breast and thigh, which is an index of higher economic returns. Earlier reports (Le Bihan-Duval et al. 1999; Jamroz et al. 2005; Murugesan et al. 2015) had shown that an increase in the proportion of eviscerated muscles (particularly the breast) is needed for increased profitability in the broiler industry. Our results agree with previous reports (Sarica and Urkmez 2016; Al-Shammari et al. 2019).

Heart weight was increased in birds fed all levels of PPPM (except 6 g/kg) in a comparable manner with the control diets (Table 8). The increased heart size recorded in the PPPM-treated birds showed that PPPM has the ability to increase the size of the heart, with resultant increase in oxygen and nutrient supply (as blood), which was reflected in the growth of the birds. Abbas et al. (2017) reported that dietary pomegranate peel powder inclusion increased heart weights of quail birds. Birds fed the 2 g/kg PPPM diet had higher but similar small intestine length with the NEGCON and POSCON birds. Birds that are fed highly nutritive diets usually have higher body weight and feed utilisation efficiency leading to improved gastrointestinal tract segments length (El-Ghousein and Al-Beitwawi 2009). In our study, PPPM<sub>2</sub> birds had improved body weight and feed utilisation efficiency compared with the POSCON birds; however this did not translate into higher small intestinal length. Our result on small intestine length agrees with the findings of Sarica and

Urkmez (2016) who reported that small intestine length of broiler birds fed 100 and 200 mg/kg PPE were not different from that of those fed the non-supplemented diets.

The 4 g/kg PPPM-supplemented groups also had improved spleen and gizzard weights compared with the NEGCON birds (Table 8). Knowledge of the weight of organs in broilers is essential in determining the health condition of the birds. A decrease in the size of the spleen (lymphoid organ) is an indication of stress and compromised immunity in birds which makes them more susceptible to pathogenic invasion (Heckert et al. 2002). It is noteworthy that the spleen weights of broilers in this study were within range reported for broiler birds by Sharifian et al. (2019), hence, the immunity of the birds may be said to not be compromised. In the studies of Sharifian et al. (2019) and Al-Shammari et al. (2019) pomegranate peel supplementation had no effect on the weight of spleen in broiler birds. Our findings on improved gizzard weight contradict those of Sharifian et al. (2019) and Al-Shammari et al. (2019).

Serum biochemical indices are the key tools used to assess the health status of animals (Hosseinizadeh et al. 2014). Total protein values were significantly improved in birds fed NEGCON, POSCON, PPPM<sub>2</sub> and PPPM<sub>6</sub> diets, whereas albumin levels were increased in the POSCON and the PPPM birds (Table 9). The improvement seen in the serum total protein and albumin had been linked to enhanced protein digestibility (Oliveira et al. 2010). Krames (2010) also associated elevated total protein levels to increased serum protein synthesis, which suggests normal liver function and enhanced growth performance. The plasma concentrations of albumin, total protein and those of serum AST which is an important intracellular enzyme are often used to assess hepatocellular injury (Hosseini-Vashan et al. 2016; Attia et al. 2015). The significant reduction in AST levels by 4 g/kg PPPM inclusion (Table 9) indicates that at this level of inclusion, PPPM had no harmful effect on liver function. Zuonongo (2013) had earlier attributed an increase in AST to hepatic cytolysis. The present results on elevated total protein and albumin levels agree with the findings of Sharifian et al. (2019) who reported that the dietary pomegranate peel extract increased the levels of these metabolites in broiler birds. However, these authors reported that dietary inclusion of pomegranate peel extract had no effect on AST levels in the birds.

Dietary supplementation of 8 g/kg PPPM was able to increase the anti-oxidative catalase enzyme activity

of broiler meat (Table 9). Medicinal plants combat lipid peroxidation in biological systems either by directly scavenging for ROS, or indirectly enhancing the innate defense mechanisms of the cells, through the activation of antioxidant enzymes like catalase (Verma et al. 2009). Catalase (CAT) is an important hepatic enzyme that provides the first line of defence to biological systems against the deleterious impact of free radicals and ROS. CAT inhibits the lipid peroxidation of hydrogen and peroxide toxicity at cellular level (Oloruntola et al. 2018d). Increase in CAT activity as observed in meat from the 8 g/kg PPPM group is an indication that the enzyme could act faster to remove free radicals in meat (Akbarian et al. 2015). The reports of Al-Shammari et al. (2019) showed that supplementation of 1% pomegranate peel and 0.5% H<sub>2</sub>O<sub>2</sub> increased the blood plasma CAT levels in broiler chickens.

## Conclusions

It can be concluded that birds fed 4 g/kg PPPM outperformed the birds that were fed  $\alpha$ -tocopherol supplemented (POSCON) diets in terms of performance, digestibility, carcass and organ indices. The effect of pomegranate peel powder meal supplementation was also pronounced in decreasing the concentration of AST at 4 g/kg and increasing the activity of catalase enzyme at 8 g/kg compared with the POSCON and NEGCON diets. In some cases, the effects of PPPM inclusion on the performance, carcass and organ indices of the birds did not follow a linear trend; hence the outcome may not be completely attributed to the positive effects of PPPM supplementation. To this end, there is need for further studies to ascertain the dietary effects of pomegranate peel powder meal on the performance, carcass and organ parameters of broiler birds.

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## Disclosure statement

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