Saudi Journal of Biological Sciences 27 (2020) 2719–2726

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

# Effect of polyphenol extract on performance, serum biochemistry, skin pigmentation and carcass characteristics in broiler chickens fed with different cereal sources under hot-humid conditions

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ARTICLE INFO

*Article history:*
Received 14 January 2020
Revised 10 June 2020
Accepted 11 June 2020
Available online 20 June 2020

*Keywords:*
Broken rice
Corn
Diet interaction
Polyphenols
Pomegranate peels
Sorghum

ABSTRACT

This study investigated the interaction between polyphenols with different cereal sources and their effects on performance, serum biochemistry, corticosterone levels and carcass characteristics in broiler chickens reared under hot-humid environment. Newly hatched coloured broiler chicks (n = 240) were randomly divided into six groups with five replicates of two different cereal sources, namely corn and broken rice-sorghum combined, and three levels of pomegranate peel polyphenol extracts (PPP) 0, 50 and 100 mg/kg in each cereal groups. Birds were maintained under standard management conditions for six weeks during hot-humid environment (Temperature: 29–36 °C; Relative Humidity: 69–80%). Fortnight body weight and feed consumption were recorded and serum biochemical constituents were estimated at 28 and 42 days of age. The body weight gain was significantly ($p < 0.05$) higher in broken rice-sorghum diet than in corn diet. The supplementation of polyphenols increased the skin and shank pigmentation. Serum protein, lipids and minerals showed significant difference due to cereals, polyphenols and their interactions. Corn-fed birds had significantly higher visceral organs weight than the alternate grain-fed broilers. The serum corticosterone levels were significantly reduced in the PPP supplemented groups than the control, it could be concluded that, supplementation of 50 mg/kg polyphenol extract from pomegranate peels improved production performance in broilers fed broken rice-sorghum as cereal source under hot-humid conditions.



## 1. Introduction

Grains are the predominant energy source not only for human population, but also are dominant in livestock and poultry production. Among the cereals, corn is used mainly in poultry feeding due its higher metabolizability, pigmentation (Dei, 2017). The corn possesses higher metabolizable energy about 13.81 MJ/kg with the presence of high pigment content which improves the colour and appearance of the birds and also enhances the value of the products (Mohamed et al., 2015). Over the years the demand–supply gap got widening due to diversion of corn for bio-fuel production and the scenario is not pleasing at the national level too. Use of alternate cereal sources is being practiced without much impact on animal production system except in poultry where the lack of pigments in those ingredients severely affects the consumer's acceptance. Irrespective of nationality, the bright coloured egg/meat is being considered more nutritious which they fetch higher returns for the producers than the use of non-pigmented cereal grains as with the case of wheat, broken rice, barley, millets, etc.

Poultry production in tropics depends on dietary anti-oxidant and anti-inflammatory properties to reducing the negative impacts of higher environmental temperature. Use of active compounds of phytogenic origin has received extensive attention due to their wide spectrum of functions. Studies with polyphenols revealed that the addition of phenolic compounds improved the performance and feed efficiency (Hassan et al., 2019). These polyphenols are mainly concentrated on the pericarp/outer protective layers of

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.06.021
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fruit or seed. Polyphenols derived from *Punica granatum* possess anti-oxidant and anti-inflammatory properties. In addition to their diversity in distribution, polyphenols interact with various biological compounds (carbohydrates, protein, plant acids, etc.), which affects their activities (Althunibat et al., 2010). Hence, the response of birds to any additives of phytogenic origin cannot be expected to be the same for different feed formulations and above mentioned reasons could be the answer for the diverse outcomes among the literatures. The effects of the diet composition on the bioavailability and biological activity of polyphenols are not clear as it involves two interactions; first interaction directly between polyphenols and components of the diet, such as binding to proteins and polysaccharides and secondly indirect interaction by the alterations of diet on gut physiology, pH, intestinal fermentations, biliary excretion and transit time. In the current study, the performance of heat stressed broilers was assessed with two-types of cereal sources supplemented with graded levels of polyphenols extracted from pomegranate peels.

## 2. Materials and methods

The protocols for the study were approved by the Institutional Animal Ethics committee (IAEC) (IAEC No. 452/01/ab/CPCSEA) with CPCSEA of ICAR-Central Avian Research Institute, Izatnagar.

### 2.1. Collection of pomegranate peels and preparation of polyphenol extract

The pomegranate peels were collected from local market fruit shops and the damaged/ or spoiled peels were removed before further processing. The peels were dried in the shade, then finely grounded with the help of grinder (SM 100; Retsch GmbH) and stored in air tight container. The polyphenols were extracted using three different solvents (aqueous, acetonitrile and methanol). The extraction procedure was carried out with grounded peels using either one of above three solvents at 1:10 ratio (one part powder to 10 part of solvent) with continuous agitation for overnight (Althunibat et al., 2010). The extracts were evaporated and the polyphenol extract (PPP) were collected and stored at 4 °C. Extracted polyphenols using all the three solvents were assessed for their *in vitro* antioxidant activity. The yield of polyphenols were comparable among the solvents but higher antioxidant activity was observed in methanolic extract. Hence, methanol was selected as solvent in the present study for polyphenols extraction and the yield was about 12 g per 100 g of dry powdered pomegranate peels. The total phenolic content was found to be 369 mg Gallic Acid Equivalent/g of dried peel extract.

### 2.2. Experimental condition, birds, design and diets

The present experiment was carried out in a 2 × 3 factorial design as three levels of PPP were supplemented in diets with two different cereal sources. A total of 240 newly hatched coloured broiler (CARIBRO DHANRAJA) chicks were randomly allocated to six treatment groups and reared for 42 days under standard management practices in hot-humid environment (Temp: 29–36 °C; RH: 69–80%). Each group consisted of five replicates of eight chicks each. The first group was fed corn-soybean meal diet containing no supplementary polyphenols. The second and third groups were fed a corn-soybean meal diet supplemented with 50 and 100 mg/kg of PPP. Fourth group was fed a broken rice-sorghum-soybean meal diet with no added PPP; fifth and sixth groups were fed a broken rice-sorghum-soybean meal based diet supplemented with 50 and 100 mg/kg PPP, respectively. The levels were determined based on earlier laboratory findings (Jose, 2014). In order to minimize the influence of dietary polyphenols low tannin containing white sorghum (0.02%) used in the present study. Birds were fed with pre-starter, starter and finisher diets formulated as per ICAR (2013) recommendations (Table 1). Fortnight body weight and feed intake were recorded and feed efficiency was determined. All mortalities were recorded as and when occurred. The skin and shank colour were assessed using the DSM's broiler colour fan at 42 days of age.

### 2.3. Serum biochemical attributes

Blood samples were collected at 28 and 42 days of feeding from 25 birds per treatment (5 per replicate) and serum was separated and stored at −20 °C till further analysis. Serum total protein content was determined by reacting the peptide bonds of protein with cupric ions at alkaline pH to form blue coloured chelate and their absorbance was measured at 578 nm. Serum albumin as a cation (pH 3.68) was bound to the anionic dye bromocresol green to form a green coloured complex whose absorbance was measured at 630 nm to determine serum albumin level and serum globulin levels were calculated by subtracting serum albumin from serum total protein. The total cholesterol content, triglyceride and HDL cholesterol were also estimated as per the standard methods (Bucolo and David 1973; Seigler and Wu, 1981; Kaplan, 1984). Serum minerals calcium and phosphorus were quantified by *O*-Cresolphthalein complexone and UV molybdate for inorganic phosphorus, respectively. The blood glucose was estimated in freshly collected sodium azide preserved plasma by glucose oxidase with formation of Quinoneimine dye at 505 nm (Folch et al., 1957). All the serum biochemical parameters were studied using the Coral® diagnostic kits, Pune, India.

### 2.4. Visceral organs, abdominal fat weights and muscle cholesterol and triglycerides

At the end of 42 days of feeding experiment, birds were sacrificed (n = 25 per treatment) *viz*., cervical dislocation. The weight of various visceral organs such as heart, spleen, bursa of Fabricius and abdominal fat were also recorded. All the weights were expressed as per cent of live body weight. The muscle lipids content of thigh muscles were extracted using Folch reagent method (Folch et al., 1957). The muscle cholesterol and triglycerides were estimated as per the standard methods (Bucolo and David, 1973; Seigler and Wu, 1981), respectively with the results were expressed as mg/dl. Serum corticosterone assay was done using ELISA Kits obtained from Labor Diagnostika Nord (LDN), Nordhorn, Germany at 42nd day of age. The blood samples were collected (n = 15 per treatment). The corticosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. Endogenous corticosterone of a serum sample competes with a corticosterone horseradish peroxidase conjugate for binding to the coated antibody. The concentration of corticosterone was measured at 450 nm using ELISA microplate reader (SpectraMax 190, Molecular Devices, U.S.A) and values were calculated from standard curve and expressed in mmol/L.

### 2.5. Statistical analysis

The data pertaining to production performance of all the birds, heat stress response and biochemical indices of 25 birds from each group were subjected to 2 × 3 factorial analysis using SPSS version 20.0 to assess the treatment effects. Where effects were significant individual treatment differences were compared using Tukey's range test.

**Table 1**
Ingredient and nutrient composition of basal diets (as fed basis, g/kg unless noted).

| Corn-soybean meal diet | | | | Broken rice-sorghum-soybean meal diet | | | |
|---|---|---|---|---|---|---|---|
| Ingredients | Pre-starter | Starter | Finisher | Ingredients | Pre-starter | Starter | Finisher |
| Maize | 549.3 | 546.5 | 578.8 | Broken Rice | 340.2 | 342.7 | 356.7 |
| Soybean meal (46%CP) | 395 | 378 | 325.8 | Sorghum | 210 | 219 | 190 |
| Rice bran oil | 21.2 | 42.4 | 58.6 | Soybean meal (46%CP) | 378 | 357 | 350 |
| Calcite | 15.4 | 15.2 | 17.3 | Calcite | 10 | 10 | 18 |
| Di-Calcium Phosphate | 9.0 | 9.5 | 11.0 | Di-Calcium Phosphate | 17 | 15 | 13 |
| Salt | 1.8 | 1.8 | 1.8 | Rice bran oil | 37 | 48 | 66 |
| L-Lysine | 3.0 | 1.5 | 1.7 | DL-Methionine | 2.7 | 2.6 | 1.9 |
| DL-Methionine | 3.0 | 2.8 | 2.7 | L-Lysine | 1.0 | 1.6 | 0.3 |
| Mineral mixture[1] | 0.15 | 0.15 | 0.15 | Mineral mixture[1] | 0.15 | 0.15 | 0.15 |
| B-Complex vitamins[2] | 0.15 | 0.15 | 0.15 | Salt | 1.8 | 1.8 | 1.8 |
| Vitamin A, $D_3$, E, K | 1.4 | 1.4 | 1.4 | B-Complex vitamins[2] | 0.15 | 0.15 | 0.15 |
| Coccidiostat[3] | 0.1 | 0.1 | 0.1 | Vitamin A, $D_3$, E, K | 1.4 | 1.4 | 1.4 |
| Toxin binder | 0.5 | 0.5 | 0.5 | Coccidiostat[3] | 0.1 | 0.1 | 0.1 |
| Total | 1000 | 1000 | 1000 | Toxin binder | 0.5 | 0.5 | 0.5 |
| | | | | Total | 1000 | 1000 | 1000 |
| *Energy and nutrient composition* | | | | | | | |
| Crude protein | 226.50 | 216.50 | 197.00 | Crude protein | 224.50 | 215.80 | 198.00 |
| Metabolizable energy, MJ/kg | 12.55 | 13.08 | 13.60 | Metabolizable energy, MJ/kg | 12.55 | 13.05 | 13.59 |
| Calcium | 9.60 | 9.50 | 9.00 | Calcium | 9.60 | 9.40 | 9.20 |
| Available Phosphorus* | 4.50 | 4.60 | 4.60 | Available Phosphorus* | 4.50 | 4.60 | 4.40 |
| Lysine* | 14.20 | 12.50 | 11.40 | Lysine* | 14.20 | 12.50 | 11.00 |
| Methionine* | 6.20 | 5.90 | 5.50 | Methionine* | 6.40 | 5.90 | 5.20 |

*Calculated values.
[1] Each 1000 g of the mineral mixture contained manganese 91 mg, zinc 91 mg, iron 85 mg, iodine 1.82 mg, copper 30.24 mg and cobalt 0.365 mg.
[2] Each kg vitamin premix contained vitamin b2 13 mg, thiamine 5 mg, pyridoxine 8 mg, niacin 320 mg, cyanocobalamine 0.05 mg, vitamin E 95 mg, calcium D-pantothenate 27.5 mg, folic acid 14 mg, calcium 30.1 mg.
[3] Coccidiostat supplied 125 mg of 3,5 dinitro-*ortho*-toluamide.

## 3. Results

### 3.1. Effect on broiler's growth performance

The body weight and weight gain showed significant ($p < 0.05$) difference due to dietary treatments (Table 2). Higher body weight and weight gain were observed in 50 mg/kg PPP supplemented broken rice-sorghum diet fed birds during the starter and finisher phases, but at pre-starter they were comparable. The supplementation of PPP (50 and 100 mg/kg) improved ($p < 0.05$) the growth performance than control. Among the cereal sources, the birds fed broken rice-sorghum had significantly ($p < 0.05$) higher body weight than corn fed group. The feed intake (Table 3) and feed efficiency (Table 4) were comparable during pre-starter and finisher phases but at starter phase (15–28 days of age) the PPP supplementation improved feed efficiency ($p < 0.05$).

### 3.2. Effect on skin and shank pigmentation

The shank and skin colour showed significant interactive effect among the treatment groups (Fig. 1). The shank and skin colour was significantly ($p < 0.05$) improved in polyphenols supplemented groups. The higher pigmentation was observed in corn fed than the rice-sorghum fed groups. However, higher ($p < 0.01$) degree pigmentation was observed in broken rice-sorghum soybean meal supplemented group than the corn fed groups.

### 3.3. Effect on serum biochemical constituents

The serum biochemical constituents showed significant difference to cereal sources, polyphenols and its interactions at various durations (28 and 42 days of age) (Table 5). The total protein, albumin, globulin and albumin: globulin content of birds remained

**Table 2**
Effect of cereal sources and pomegranate peel polyphenol extracts on body weight and weight gain, g in broilers.

| Cereal source | Polyphenol levels | Overall weight | Pre-starter gain | Starter gain | Finisher gain | Overall gain |
|---|---|---|---|---|---|---|
| Diet matrix × Polyphenol levels interactions | | | | | | |
| Corn | 0 | 1736 ± 17[c] | 327 ± 4 | 599 ± 13[b] | 799 ± 12[c] | 1695 ± 18[c] |
| | 50 | 1827 ± 18[b] | 313 ± 5 | 669 ± 12[a] | 841 ± 16[b] | 1787 ± 16[b] |
| | 100 | 1846 ± 22[b] | 314 ± 7 | 671 ± 16[a] | 864 ± 15[b] | 1804 ± 19[b] |
| Broken rice-sorghum | 0 | 1834 ± 20[b] | 311 ± 5 | 641 ± 15[b] | 820 ± 16[bc] | 1793 ± 19[b] |
| | 50 | 1900 ± 24[a] | 311 ± 6 | 697 ± 15[a] | 911 ± 18[a] | 1858 ± 17[a] |
| | 100 | 1828 ± 23[b] | 312 ± 6 | 699 ± 17[a] | 830 ± 17[b] | 1786 ± 20[b] |
| P-value | | 0.001 | 0.107 | 0.001 | 0.001 | 0.001 |
| Effect of cereal sources | | | | | | |
| Corn | | 1803[b] | 319 | 647[b] | 834[b] | 1768[b] |
| Broken rice-sorghum | | 1854[a] | 312 | 699[a] | 854[a] | 1813[a] |
| P-value | | 0.003 | 0.154 | 0.001 | 0.044 | 0.004 |
| Effect of polyphenol levels | | | | | | |
| 0 | | 1823 | 321 | 639[b] | 831[b] | 1787[b] |
| 50 | | 1830 | 313 | 696[a] | 857[a] | 1798[a] |
| 100 | | 1837 | 313 | 683[a] | 847[a] | 1800[a] |
| P-value | | 0.098 | 0.298 | 0.001 | 0.041 | 0.024 |

[a,b,c] Within in a column, means with different superscripts differ significantly ($P \leq 0.05$).

**Table 3**
Effect of cereal sources and pomegranate peels polyphenol extract on feed intake, g per bird in broilers.

| Cereal source | Polyphenol levels | Pre-starter | Starter | Finisher | Overall period |
|---|---|---|---|---|---|
| Diet matrix × Polyphenol levels interactions | | | | | |
| Corn | 0 | 477.60 ± 4.32 | 1069.05 ± 39.18 | 1919.05 ± 112.16 | 3465.22 ± 107.79 |
| | 50 | 469.90 ± 5.66 | 1116.93 ± 37.00 | 1985.43 ± 106.26 | 3570.17 ± 118.73 |
| | 100 | 473.58 ± 3.96 | 1134.97 ± 35.26 | 1900.71 ± 119.24 | 3599.54 ± 103.30 |
| Broken rice-sorghum | 0 | 482.73 ± 6.01 | 1086.21 ± 72.68 | 1959.94 ± 99.80 | 3528.53 ± 127.20 |
| | 50 | 487.50 ± 10.69 | 1162.23 ± 44.84 | 2028.95 ± 85.97 | 3678.27 ± 74.99 |
| | 100 | 485.97 ± 8.19 | 1156.38 ± 97.82 | 2027.68 ± 107.14 | 3670.39 ± 100.70 |
| P-value | | 0.418 | 0.273 | 0.787 | 0.434 |
| Effect of cereal sources | | | | | |
| Corn | | 473.05 | 1106.98 | 1965.06 | 3545.10 |
| Broken rice-sorghum | | 485.40 | 1134.94 | 2005.52 | 3625.86 |
| P-value | | 0.195 | 0.106 | 0.981 | 0.611 |
| Effect of polyphenol levels | | | | | |
| | 0 | 480.17 | 1077.63 | 1939.50 | 3497.29 |
| | 50 | 477.74 | 1139.58 | 2007.19 | 3624.51 |
| | 100 | 479.78 | 1145.68 | 2009.20 | 3634.65 |
| P-value | | 0.221 | 0.236 | 0.586 | 0.277 |

**Table 4**
Effect of cereal sources and pomegranate peels polyphenol extracts on feed efficiency in broilers.

| Cereal source | Polyphenol levels | Pre-starter | Starter | Finisher | Overall period |
|---|---|---|---|---|---|
| Diet matrix × Polyphenol levels interactions | | | | | |
| Corn | 0 | 1.46 ± 0.03 | 2.12 ± 0.06[a] | 2.34 ± 0.37 | 2.02 ± 0.19 |
| | 50 | 1.42 ± 0.07 | 1.67 ± 0.05[b] | 2.17 ± 0.08 | 1.86 ± 0.04 |
| | 100 | 1.53 ± 0.04 | 1.75 ± 0.07[b] | 2.20 ± 0.15 | 1.95 ± 0.11 |
| Broken rice-sorghum | 0 | 1.55 ± 0.14 | 1.60 ± 0.08[b] | 2.17 ± 0.28 | 1.98 ± 0.22 |
| | 50 | 1.57 ± 0.13 | 1.61 ± 0.08[b] | 2.26 ± 0.21 | 1.88 ± 0.13 |
| | 100 | 1.56 ± 0.09 | 1.77 ± 0.13[ab] | 2.42 ± 0.26 | 2.01 ± 0.16 |
| P-value | | 0.807 | 0.001 | 0.481 | 0.299 |
| Effect of cereal sources | | | | | |
| Corn | | 1.47 | 1.85[a] | 2.37 | 2.04 |
| Broken rice-sorghum | | 1.56 | 1.66[b] | 2.31 | 1.99 |
| P-value | | 0.215 | 0.035 | 0.776 | 0.665 |
| Effect of polyphenol levels | | | | | |
| 0 | | 1.51 | 1.86[a] | 2.45 | 2.05 |
| 50 | | 1.49 | 1.64[b] | 2.22 | 1.87 |
| 100 | | 1.55 | 1.76[ab] | 2.36 | 2.03 |
| P-value | | 0.839 | 0.040 | 0.621 | 0.200 |

[a,b,c] Within in a column, means with different superscripts differ significantly ($P \leq 0.05$).

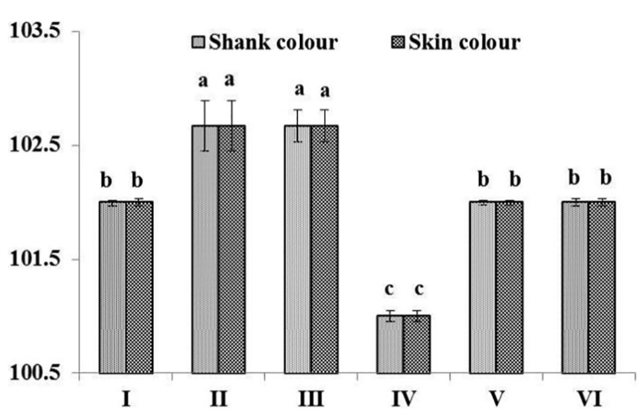


**Fig. 1.** Effect of cereal sources and pomegranate peel polyphenol extracts on shank and skin colour in broilers. [a,b,c] Within in a column, means with different superscripts differ significantly ($P \leq 0.05$).

unaffected ($p > 0.05$) due to interaction, cereal sources or PPP levels. There was an age dependent reduction ($p < 0.05$) in the concentration of total protein, albumin and globulin was observed. The serum total cholesterol, high density lipoprotein cholesterol (HDL-cholesterol) and triglycerides showed significant difference among the treatments (Table 6). The total and HDL-cholesterol contents varied with choice of cereal sources, corn fed groups recorded higher ($p < 0.05$) values. Addition of PPP did not alter the total and HDL-cholesterol content in both cereal types. Similarly, the serum triglycerides content also showed a reduction in the concentration due to supplementation of PPP irrespective of the cereal sources. Unlike total or HDL-cholesterol, the triglycerides was not affected ($p > 0.05$) either by corn or broken rice-sorghum feeding. The serum calcium content was significantly ($p < 0.05$) higher in corn as cereal source fed birds than the alternate combined-cereal source (Table 7). However, calcium levels showed significant difference due to cereal-polyphenols interaction but not due to polyphenols levels.

Higher calcium levels were recorded at 42 days (10.35) of feeding than at 28 days (8.08 mg/dl). Serum inorganic phosphorus and uric acid levels did not show any response due to cereals sources, polyphenols levels as well as the duration of feeding. The uric acid content was higher in corn groups and also during the early growing stages. The plasma glucose level showed a dose dependent reduction due to PPP supplementation in both the cereal types used in the study. The higher glucose concentration was observed in corn fed groups than the broken rice-sorghum and also a PPP dose dependent reduction in broilers.

### 3.4. Effect on visceral organs, muscle cholesterol and triglycerides

Dietary variations both individual and interactions had influence on weight of giblets, spleen, bursa of Fabricius, abdominal

**Table 5**
Effect of cereal sources and pomegranate peels polyphenol extracts on serum protein, g/dL content in broilers.

| Days | Cereal sources | Polyphenol levels | Total protein | Albumin | Globulin | A:G ratio* |
|---|---|---|---|---|---|---|
| Diet matrix × Polyphenol levels × Period interactions | | | | | | |
| 28 | Corn | 0 | 5.28 ± 0.13 | 3.13 ± 0.16 | 2.15 ± 0.24 | 1.77 ± 0.43 |
| | | 50 | 5.32 ± 0.25 | 3.08 ± 0.10 | 2.24 ± 0.26 | 1.59 ± 0.27 |
| | | 100 | 6.02 ± 0.38 | 3.02 ± 0.20 | 3.01 ± 0.48 | 1.21 ± 0.23 |
| | Broken rice -sorghum | 0 | 5.46 ± 0.12 | 2.91 ± 0.15 | 2.54 ± 0.21 | 1.23 ± 0.16 |
| | | 50 | 5.81 ± 0.20 | 3.69 ± 0.26 | 2.11 ± 0.20 | 2.10 ± 0.56 |
| | | 100 | 5.85 ± 0.12 | 3.79 ± 0.18 | 2.06 ± 0.20 | 1.84 ± 0.28 |
| 42 | Corn | 0 | 4.25 ± 0.18 | 2.52 ± 0.10 | 1.73 ± 0.20 | 1.64 ± 0.27 |
| | | 50 | 4.39 ± 0.16 | 2.47 ± 0.10 | 1.92 ± 0.17 | 1.39 ± 0.18 |
| | | 100 | 4.29 ± 0.17 | 2.34 ± 0.10 | 1.95 ± 0.17 | 1.28 ± 0.14 |
| | Broken rice-sorghum | 0 | 3.95 ± 0.14 | 2.30 ± 0.09 | 1.64 ± 0.12 | 1.46 ± 0.13 |
| | | 50 | 4.28 ± 0.18 | 2.54 ± 0.05 | 1.74 ± 0.17 | 1.54 ± 0.13 |
| | | 100 | 4.00 ± 0.09 | 2.40 ± 0.09 | 1.60 ± 0.15 | 1.69 ± 0.32 |
| P- value | | | 0.217 | 0.417 | 0.261 | 0.655 |
| Effect of cereal sources | | | | | | |
| Corn | | | 4.92 | 2.76 | 2.16 | 1.48 |
| Broken rice-sorghum | | | 4.82 | 2.87 | 1.95 | 1.64 |
| P- value | | | 0.373 | 0.174 | 0.110 | 0.334 |
| Effect of polyphenol levels | | | | | | |
| 0 | | | 4.73 | 2.71 | 2.02 | 1.52 |
| 50 | | | 4.95 | 2.95 | 2.00 | 1.66 |
| 100 | | | 4.94 | 2.79 | 2.16 | 1.50 |
| P -value | | | 0.206 | 0.073 | 0.583 | 0.714 |
| Effect of period | | | | | | |
| 28 | | | 5.55[a] | 3.20[a] | 2.35[a] | 1.62 |
| 42 | | | 4.19[b] | 2.43[b] | 1.76[b] | 1.50 |
| P- value | | | 0.001 | 0.001 | 0.001 | 0.466 |

*A:G ratio: albumin to globulin ratio.
[a,b,c] Within in a column, means with different superscripts differ significantly ($P \leq 0.05$).

**Table 6**
Effect of cereal sources and pomegranate peels polyphenol extracts on serum lipid profile, mg/dL in broilers.

| Days | Cereal sources | Polyphenol levels | Total cholesterol | HDL-cholesterol | Triglycerides |
|---|---|---|---|---|---|
| Diet matrix × PPP levels × period interactions | | | | | |
| 28 | Corn | 0 | 91.59[a] ± 2.10 | 27.24[c] ± 0.93 | 122.69[a] ± 7.74 |
| | | 50 | 88.86[ab] ± 2.57 | 27.92[c] ± 2.10 | 118.12[ab] ± 4.68 |
| | | 100 | 86.14[ab] ± 3.20 | 28.18[c] ± 0.83 | 116.19[abc] ± 2.91 |
| | Broken rice -sorghum | 0 | 79.44[ab] ± 1.85 | 21.80[c] ± 0.33 | 118.57[ab] ± 5.11 |
| | | 50 | 77.46[ab] ± 1.06 | 23.64[c] ± 0.60 | 108.73[abc] ± 1.86 |
| | | 100 | 79.09[ab] ± 2.15 | 26.90[c] ± 1.77 | 113.41[abc] ± 1.49 |
| 42 | Corn | 0 | 85.40[ab] ± 1.72 | 37.98[b] ± 2.41 | 99.26[bc] ± 4.02 |
| | | 50 | 81.17[ab] ± 1.82 | 44.92[a] ± 1.31 | 98.17[bc] ± 4.60 |
| | | 100 | 71.18[b] ± 2.99 | 42.79[ab] ± 0.80 | 102.33[abc] ± 4.21 |
| | Broken rice-sorghum | 0 | 74.95[ab] ± 1.02 | 37.92[b] ± 1.07 | 103.42[abc] ± 6.26 |
| | | 50 | 77.46[ab] ± 1.45 | 40.24[ab] ± 0.76 | 96.09[c] ± 2.91 |
| | | 100 | 72.57[ab] ± 2.44 | 44.11[ab] ± 1.64 | 96.74[c] ± 3.05 |
| P- value | | | 0.012 | 0.001 | 0.001 |
| Effect of cereal sources | | | | | |
| Corn | | | 83.00[a] | 34.84[a] | 109.40 |
| Broken rice-sorghum | | | 76.83[b] | 32.43[b] | 106.16 |
| P- value | | | 0.010 | 0.003 | 0.199 |
| Effect of polyphenol levels | | | | | |
| 0 | | | 84.10 | 32.95[b] | 110.98[a] |
| 50 | | | 78.22 | 33.95[ab] | 107.17[ab] |
| 100 | | | 82.00 | 36.01[a] | 105.28[b] |
| P –value | | | 0.404 | 0.049 | 0.013 |
| Effect of period | | | | | |
| 28 | | | 82.73 | 25.95[b] | 116.38[a] |
| 42 | | | 78.15 | 41.33[a] | 99.34[b] |
| P- value | | | 0.058 | 0.001 | 0.001 |

[a,b,c] Within in a column, means with different superscripts differ significantly ($P \leq 0.05$).

fat, muscle cholesterol and triglycerides content (Table 8). The weight of giblet, spleen and bursa of Fabricius were reduced ($p < 0.01$) by the incorporation of polyphenols and their effect was more pronounced with broken rice-sorghum based diets. Similarly, the PPP supplementation reduced the weight of giblet and spleen but not the bursa of Fabricius. However, the abdominal fat (%) were neither affected ($p > 0.05$) by the cereal sources nor by the PPP levels. Similar to the serum studies, the muscle cholesterol and triglycerides contents were reduced by the PPP supplementation both in interaction as well as PPP effect. However, the birds fed with alternate cereal sources (broken rice-sorghum fed group) had higher ($p < 0.05$) muscle total cholesterol than the corn fed groups. The circulating corticosterone (mmol/L) concentration was significantly ($p < 0.05$) reduced by the supplementation of PPP in both cereal sources studied. Serum corticosterone significantly lower in alternate cereal group when compared to corn based diet

**Table 7**
Effect of cereal sources and pomegranate peels polyphenol extracts on serum calcium, phosphorus, uric acid and plasma glucose, mg/dL levels in broilers.

| Days | Cereal sources | Polyphenol levels | Calcium | Phosphorus | Uric acid | Plasma glucose |
|---|---|---|---|---|---|---|
| Diet matrix × PPP levels × period interactions | | | | | | |
| 28 | Corn | 0 | 8.58 ± 0.58[bcd] | 5.61 ± 0.08 | 5.60 ± 0.46 | 389.27 ± 4.28[a] |
| | | 50 | 8.10 ± 0.29 [cd] | 6.03 ± 0.45 | 5.09 ± 0.34 | 357.21 ± 5.87[ab] |
| | | 100 | 8.71 ± 0.99[bcd] | 5.82 ± 0.51 | 6.16 ± 0.52 | 340.81 ± 4.35[b] |
| | Broken rice -sorghum | 0 | 7.62 ± 0.30[d] | 5.26 ± 0.27 | 5.56 ± 0.17 | 387.58 ± 3.64[a] |
| | | 50 | 7.30 ± 0.31[d] | 5.06 ± 0.20 | 5.16 ± 0.18 | 371.98 ± 5.35[a] |
| | | 100 | 8.18 ± 0.55 [cd] | 4.71 ± 0.59 | 4.86 ± 0.02 | 335.28 ± 9.07[b] |
| 42 | Corn | 0 | 10.82 ± 0.46[ab] | 4.87 ± 0.08 | 4.46 ± 0.22 | 379.27 ± 4.28[a] |
| | | 50 | 10.24 ± 0.06[abc] | 5.74 ± 0.48 | 4.26 ± 0.13 | 361.21 ± 5.87[ab] |
| | | 100 | 12.10 ± 0.82[a] | 4.37 ± 0.18 | 4.44 ± 0.17 | 340.81 ± 4.35[b] |
| | Broken rice-sorghum | 0 | 10.65 ± 0.56[ab] | 4.75 ± 0.38 | 4.26 ± 0.07 | 387.58 ± 3.64[a] |
| | | 50 | 10.24 ± 0.07[abc] | 5.56 ± 0.71 | 4.36 ± 0.17 | 361.98 ± 5.35[ab] |
| | | 100 | 10.03 ± 0.03[abc] | 7.20 ± 1.08 | 4.10 ± 0.04 | 325.28 ± 9.07[c] |
| P- value | | | 0.183 | 0.022 | 0.264 | 0.016 |
| Effect of Cereal Sources | | | | | | |
| Corn-Soybean | | | 9.76[a] | 5.41 | 5.00[a] | 395.77[a] |
| Broken rice-sorghum-soybean | | | 9.02[b] | 5.46 | 4.72[b] | 364.95[b] |
| P- value | | | 0.012 | 0.958 | 0.048 | 0.038 |
| Effect of PPP levels | | | | | | |
| 0 | | | 9.42 | 5.12 | 4.97 | 388.42[a] |
| 50 | | | 8.97 | 5.60 | 4.72 | 364.60[b] |
| 100 | | | 9.76 | 5.53 | 4.89 | 328.05[c] |
| P –value | | | 0.096 | 0.358 | 0.370 | 0.018 |
| Effect of period | | | | | | |
| 28 | | | 8.08[b] | 5.42 | 5.40[a] | 388.36 |
| 42 | | | 10.68[a] | 5.49 | 4.31[b] | 361.56 |
| P- value | | | 0.001 | 0.999 | 0.001 | 1.000 |

[a,b,c] Within in a column, means with different superscripts differ significantly ($P \leq 0.05$).

**Table 8**
Effect of cereal sources and pomegranate peels polyphenol extracts on visceral organs, abdominal fat, % BW, muscle cholesterol and triglycerides, mg/dL and serum corticosterone, mMol/L in broilers.

| Cereal sources | Polyphenol levels | Giblet | Spleen | Bursa of Fabricius | Abdominal fat | Muscle | | Serum Corticosterone |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Cholesterol | Triglycerides | |
| Diet matrix × PPP levels interactions | | | | | | | | |
| Corn | 0 | 88.00 ± 7.21[a] | 2.75 ± 0.56[a] | 1.77 ± 0.40[c] | 21.25 ± 1.76[b] | 110.68 ± 6.14[a] | 155.32 ± 5.67[a] | 143.25 ± 3.41[a] |
| | 50 | 80.25 ± 3.19[ab] | 0.88 ± 0.08[bc] | 2.50 ± 0.42[bc] | 29.25 ± 3.89[ab] | 96.04 ± 3.14[bc] | 154.35 ± 4.69[a] | 109.33 ± 5.37[b] |
| | 100 | 78.25 ± 3.70[ab] | 0.70 ± 0.12[c] | 3.00 ± 0.01[abc] | 31.50 ± 3.55[a] | 91.21 ± 5.14[c] | 108.03 ± 7.68[c] | 79.05 ± 1.79[d] |
| Broken rice-sorghum | 0 | 71.50 ± 3.31[bc] | 2.00 ± 0.38[ab] | 4.00 ± 0.46[a] | 23.75 ± 0.86[ab] | 105.19 ± 3.14[ab] | 130.72 ± 3.47[b] | 118.04 ± 3.80[b] |
| | 50 | 62.50 ± 1.95[c] | 1.25 ± 0.16[bc] | 3.25 ± 0.31[ab] | 24.00 ± 2.70[ab] | 111.14 ± 4.14[a] | 135.85 ± 4.57[ab] | 91.08 ± 3.90[c] |
| | 100 | 64.25 ± 0.73[c] | 1.00 ± 0.00[bc] | 2.50 ± 0.19[bc] | 22.25 ± 0.94[b] | 103.32 ± 1.14[ab] | 127.12 ± 4.17[bc] | 72.41 ± 2.92[d] |
| P-value | | 0.001 | 0.001 | 0.001 | 0.043 | 0.031 | 0.023 | 0.001 |
| Effect of cereal sources | | | | | | | | |
| Corn | | 82.17[a] | 1.44 | 2.42[b] | 27.33 | 99.31[b] | 139.32 | 117.21[a] |
| Broken rice-sorghum | | 66.08[b] | 1.42 | 3.25[a] | 23.33 | 106.55[a] | 131.23 | 93.84[b] |
| P-value | | 0.001 | 0.916 | 0.005 | 0.064 | 0.035 | 0.204 | 0.001 |
| Effect of PPP levels | | | | | | | | |
| 0 | | 79.75[a] | 2.38[a] | 2.88 | 22.50 | 107.94[a] | 143.02[a] | 129.64[a] |
| 50 | | 71.375[b] | 1.06[b] | 2.88 | 26.63 | 103.59[ab] | 145.10[a] | 100.20[b] |
| 100 | | 71.25[b] | 0.85[b] | 2.75 | 26.88 | 97.27[b] | 117.56[b] | 75.73[c] |
| P-value | | 0.054 | 0.001 | 0.908 | 0.174 | 0.039 | 0.001 | 0.001 |

(93.8 *vs.* 117.2 mMol/L). A dose dependent reduction ($p < 0.05$) in the concentration of serum corticosterone was observed.

## 4. Discussion

### 4.1. Effect on bird's performance

Higher body weight among rice-sorghum based diet fed birds than corn based diet indicated better utilization of nutrients. The supplementary levels of 50 and 100 mg/kg PPP showed better growth performance than control. The findings had underlined the observations that broilers could be fed with dehulled rice as sole cereal source to achieve higher final weight than corn fed groups (Mašek et al., 2014). Similar to the present findings, broilers were fed with phenolic compound (tannic acid) in combination with corn-soybean meal diet and observed significantly higher final body weight, weight gain and feed utilization compared to the control and thymol and gallic acid supplemented groups (Tester et al., 2006). The ability of tannic acid's to reduce ceacal pH significantly was attributed to be the reason behind the higher growth performance. Even though, the present findings showed a similar trend among both the cereals, a more pronounced effect was observed with rice-sorghum based diet.

Improved performance in broken rice-sorghum supplemented diet could be due to following reasons; the rice has higher starch and lower fibre content, that stimulate relatively higher glucose and insulin postprandial responses and which might have increased the bird's feed consumption and in turn the weight gain. The broilers fed with dehulled rice were observed to have similar findings and the author elaborates smaller particle size and low structural carbohydrates content were the reason behind this significance (Tawfeek et al., 2014).

Recent publications repeatedly confirmed the role of dietary anti-oxidants in improving the performance of heat stress broilers (Hosseini-Vashan et al., 2016). The active principles of various plant extracts also revealed similar findings under high environmental temperature conditions. Dried tomato pomace (as source of polyphenols) fed broilers were reported to have reduction in the detrimental effects of heat stress in them due to its anti-oxidant property (Guajardo-Flores et al., 2006). Moreover, the supplementation of polyphenols through the diet could be more valuable in broiler chickens exposed to high temperature (Mazur-Kuśnirek et al., 2019). The presence of higher anti-oxidant activity due to its higher phenolic content in sorghum grains could exhibit an additive effect with the supplemented polyphenols compared with the pigment corn grain which exhibits only moderate activity (Rajput et al., 2012). The performance was similar for both the levels of supplementation (50 and 100 mg/kg) were comparable, a dose of 50 mg/kg will be sufficient.

Similar to the present findings of insignificant variation in feed intake and feed efficiency, no significant ($p > 0.05$) effect was observed in broilers fed grape pomace concentrate and green tea polyphenols (Brenes et al., 2008; Lipiński et al., 2017). Supplemented polyphenols did not supply protein or energy to the broilers; however, due to their antioxidant activity there was numerical improvement in feed utilization. The improvement in production performance in supplemented groups could be attributed to the combination increase in feed consumption and reduction in environmental stress.

### 4.2. Effect on skin pigmentation

Higher pigmentation was observed in shank and skin pigmentation in supplemented (50 and 100 mg/kg PPP) broilers fed rice-sorghum diet. This indicated their pigmentation potential on birds reared with non-pigmented grains. This might be due to absence of pigments in those cereal grains, hence incorporation of PPP has outward effect on the colour development. The improvement in the skin and shank colour may be attributed to the flavonoids and other pigmented contents in the pomegranate peel extracts. The exogenous incorporation of pigments (both natural and synthetic pigments) improved the appearance of both skin/shank and egg yolk colours. In a study with natural pigment marigold, an increase in feed intake, shank and carcass colours than the control (Attah, 2002) was reported. Similar results were obtained while feeding combination of synthetic carotenoids in broilers (El-Damrawy, 2014).

### 4.3. Effect on serum biochemistry

Serum total protein and globulin content were increased significantly with increase in dietary PPP levels, with high concentration ($p < 0.01$) was observed when PPP was supplemented at 100 mg/kg in corn-soybean diet. The effect of polyphenols on serum protein profile disappeared during the finisher phase (29–42 days) of feeding. This variation may be due to less polyphenols intake with respect to body size. Surprisingly, significant ($p < 0.01$) higher protein and globulin content was observed in the corn fed birds than the broken rice-sorghum diets. This might be due to higher protein content in corn than rice and also the presence of anti-nutritional factor, tannin in sorghum. In a study, there was significant reduction in protein digestion in sorghum fed white leghorn birds than the control (Teissedre and Waterhouse, 2000). However, the albumin content at both 28 and 42 days was significantly higher in broken-rice sorghum diet with 50 mg/kg PPP than all the three levels in corn fed birds. The albumin: globulin ratio too followed similar trend as that of albumin but no significance difference was observed at 42 days of feeding. The serum total protein is an indicative of protein quality fed to the birds. Similar finding of higher serum total protein in alternate grain feeding was observed in broiler chickens fed with sorghum and millets.

Similar to present findings, a reduction in circulating levels of triglycerides and HL ratio was observed in heat stressed broilers fed with 100 and 200 mg/kg grape seed extract (Aengwanich and Suttajit, 2010). The positive effect of polyphenols on serum lipid indices could be attributed towards the negative correlation between total phenolic compounds and low density lipoproteins peroxidation in humans (Akbari and Torki, 2013). The findings of the study is further strengthened by the reports that the high concentration of anti-oxidants halts the peroxidation process and reduces the circulating triglycerides levels (Akbari and Torki, 2013). While looking at cereal sources perspective, there was increase in serum total cholesterol and reduction in triglycerides levels among commercial broilers fed with graded levels of finger millets as a replacement of corn. The proanthocyanidins in sorghum, in addition to its anti-oxidative property, were also reported to possess cholesterol lowering effects (Dykes and Rooney, 2006).

The circulatory uric acid levels remained unaffected by the interactions and polyphenols but the corn fed bird had significantly higher ($p < 0.05$) levels than the rice-sorghum fed groups. The reduction in circulating levels of uric acid in broken rice-sorghum fed group is due to the presence of proanthocyanidins which promotes urinary tract health in broilers. The plasma glucose levels were significantly reduced by polyphenol supplementation in both cereal groups. The hypoglycemic property was attributed to the inhibition of serum glucose transporter I (SGLT I) activity by the galloyl residues epicatechin gallate and epigallocatechin gallate contents in the polyphenolic extracts. The tannin from sorghum has the property to bind with carbohydrates especially to starch numerically reduced the plasma glucose levels but their effect was not prominently observed in the present study.

### 4.4. Visceral organs weight, muscle cholesterol and triglycerides levels

Among the cereal sources, corn fed birds had significantly ($p < 0.01$) higher visceral organ weight than the alternate grain fed broilers. Similar trend was found in spleen, bursa of Fabricius as well but no cereal and PPP effects were observed in both organs respectively. There was no significance/acceptable reasoning for changes in internal organs weight could be traced out. However, the abdominal fat content showed irregular effect but the amount of fat content was significantly ($p < 0.05$) less in broken rice-sorghum diet supplemented with 100 mg/kg PPP than its corn counterpart. The muscle cholesterol and triglycerides content were reduced ($p < 0.05$) by cereal $\times$ polyphenols interactions. The muscle cholesterol content was higher in broken rice-sorghum and graded levels of polyphenols incorporated diets, which followed the similar as that of serum cholesterols.

The muscle triglycerides showed only polyphenols effect but no cereal grain of choice effect. The feeding of broken rice-sorghum as cereal source augmented the negative effects of heat stress and improved the production performance, biochemical attributes in broilers reared under high environmental temperature. Sorghum contains higher protein, essential amino acids and trace minerals than corn which could be exploited. Pomegranate peel polyphenols at 50 mg/kg improved the performance, biochemical attributes and pigmentation which could fetch higher consumer's preference for broilers fed with non-pigmented cereals. Similarly, Mahmoud et al. (2015) revealed a significant decrease in the serum corticosterone levels in heat stressed broilers after supplementation of polyphenols derived from Propolis. EOF%, an indicator of oxidative stress in the erythrocyte membranes was reduced in present study which might be due to antioxidant property of PPP. The incorporation of PPP (both 50 and 100 mg/kg) significantly reduced this stress marker serum corticosterone irrespective of the cereal sources. Feeding 100 and 200 mg/kg grape seed extract to heat stressed broilers, significantly reduced heterophils: lymphocytes ratio an another stress indicator (Aengwanich and Suttajit, 2010). Polyphenols extracted from tamarind seeds reduced the negative effects of heat stress in broilers (Dykes and Rooney, 2006). This positive effect was attributed to the anti-oxidant property of polyphenols extract.

## 5. Conclusion

Pomegranate peel polyphenols at 50 mg/kg improved the performance, biochemical attributes and pigmentation which could fetch higher consumer's preference for broilers fed with non-pigmented cereals.

## Conflict of interest

The authors declare that there is no conflict of interest.

## Acknowledgments

The authors are thankful to Director, ICAR-CARI, Izatnagar and ICAR-IVRI, Izatnagar, India for providing necessary facilities to carryout the work.

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