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Effect of Supplementation of Pomegranate (*Punica Granatum*) Peel Powder and Multienzyme on Performance of Broiler Chickens

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ABSTRACT

The goal of the current study was to determine the effect of supplementation of pomegranate (*Punica granatum*) peel powder (PPP) and multienzyme to broiler chickens' diets on growth performance, carcass characteristics, nutrient digestibility, nitrogen balance and haemato-biochemical parameters. One hundred sixty, day-old Ven cobb 430 broiler chicks were randomly assigned to four treatment groups (T1 to T4), each consisting of four replicates of ten chicks. The feeding trial was carried out using a broiler starter (1–21 days) and finisher (22–35 days) ration. Birds were offered basal feed as per the BIS (2007). The control group (T1) received only a non-supplemented basal diet, while groups T2, T3 and T_4 received 0.5% PPP, 0.05% multienzyme and 0.5% PPP + 0.05% multi-enzyme in their basal feed, respectively. After the feeding study, one bird per replicate from each treatment was randomly selected for a 5-day metabolism trial to assess nitrogen balance and Metabolizable nutrients. At the end of the experiment, blood samples were collected for blood and serum analysis, and one bird per replicate was sacrificed to evaluate carcass traits and meat quality parameters. The supplementation of PPP and multi-enzyme alone and in combination significantly ($P<0.01$) improved body weight, body weight gain, feed conversion ratio, performance index, protein efficiency ratio, dry matter, ether extract and crude protein metabolisability and nitrogen balance. Significant ($P<0.05$) improvement was observed on feed consumption. However, dietary inclusion did not affect dressed weight, eviscerated weight, weight of heart, liver, gizzard, giblet, intestine length of broiler birds. Therefore, on the basis of results obtained, the present study revealed that the inclusion of PPP @ 0.5% and multienzyme @ 0.05% enhanced the overall performance of broilers.

KEYWORDS: Broilers, Carcass, Growth Performance, Multienzyme, Pomegranate peel powder.

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INTRODUCTION

Poultry is one of the fastest growing sectors globally with 35 per cent of the global meat production in 2020, chicken meat showed the largest growth in absolute and relative terms since 2000 (104 per cent or 61 million tonnes) and was the most produced type of meat in 2020 (FAO, 2022). Poultry is one of the agricultural sectors in India that is expanding the fastest. India ranked third in the world for producing eggs, sixth for producing chicken meat and fifth for producing poultry (FAO, 2022). The growth of poultry meat production has increased by 6.86% over previous year i.e. 2021 (Basic Animal Husbandry Statistics, DAHD 2022).

Chicken meat has emerged as one of the cheapest sources of excellent quality protein. Because of worries about antibiotic residues in tissue and the development of bacterial resistance, the use of antibiotics as growth promoters in chicken has been outlawed. These concerns prompted a recent examination into various feed additives as potential replacements for feed antibiotics (Ramteke et al., 2022). Feed price constitutes around 80 per cent of the total production cost (National Action Plan for Egg and Poultry, 2022).

In recent years, broiler production has shown a growing interest in using phytobiotics as natural growth-promoting feed additives (Ramteke et al., 2022). Pomegranate Peel (PP) is a by-product that is

rich in many high molecular weight polyphenol, such as flavonoids (flavanols as catechin, epicatechin and gallocatechin as well as anthocyanins), phenolic acids (gallic, ellagic, caffeic, citric, tartaric, malic and ascorbic), hydrolyzable tannins (ellagitannins, punicalin, punicalagin, pedunculagin and gallotannins) and condensed tannins (Kandylis and Kokkinomagoulos, 2020). Besides, pomegranate and its by-product showed antimicrobial, anticarcinogenic, antioxidant, anti-inflammatory and growth promoting effects (Reddy et al., 2007; Ghasemi-Sadabadi et al., 2021 and Elnaggar et al., 2022). Poultry growth performance metrics are significantly impacted by peel powder, pomegranate powder (PPP), pomegranate peel extract (PPE) and pomegranate peels pomace.

Enzymes are mineral, vitamin and amino acid-based biological catalysts. They do not alter, but they do cause biochemical reactions. Non-Starch Polysaccharides (NSPs) such as cellulose, lignin, arabinoxylan (wheat and oat) and phytase are present in the majority of feed ingredients used in poultry diets at varying amounts (Parsippany, 2008). Supplementation of broiler feeds with multienzyme preparations resulted in improved body weight gain (BWG) and feed efficiency (Daskiran et al., 2004). Enzyme supplementation in feed plays a crucial role in enhancing nutrient availability and mitigating the negative effects of anti-nutritional factors present in feed ingredients. Therefore, the present study was conducted on broiler chickens to observe the effect of supplementation of PPP and multienzyme on growth performance, nutrient digestibility, nitrogen balance, hemato-biochemical parameters and economics of broiler chickens production

MATERIALS AND METHODS

Experimental birds and diet

A total of 160 day-old, unsexed, healthy Ven Cobb-430Y broiler chicks were procured and vaccinated against Ranikhet disease (day 7) and IBD (day 14). Chicks were individually weighed and randomly allotted to four treatment groups (40 chicks each), with four replicates of 10 chicks per group. ISO-certified basal starter and finisher feeds were procured as per BIS (2007) guidelines. Feed supplements (PPP and a commercial multienzyme from Adelbert Vegyszerek Pvt. Ltd., Kanpur) were procured locally and incorporated into the basal diets. Four different treatment diets were prepared for the feeding of broilers under different dietary groups. The treatment groups were as follows: T1 (control) received the basal diet; T2 received the basal diet supplemented with 0.5% PPP; T3 received the basal diet supplemented with 0.05% multienzyme; and T4 received the basal diet supplemented with both 0.5% PPP and 0.05% multienzyme. The proximate nutrient composition of basal feed and PPP has been presented in Table 1.

Feeding trial

A 35-day feeding trial was conducted from October 20 to November 23, 2024, during which broiler starter diets were fed up to 21 days and finisher diets up to 35 days as per BIS (2007) and treatment protocols. Feed and water were provided ad libitum, and daily group-wise feed intake was recorded. Following the feeding trial, a 5-day metabolism trial was conducted using one chick per replicate from each treatment to assess nutrient digestibility and nitrogen balance. One broiler per replicate was sacrificed at the end of the experiment for carcass evaluation.

Table 1. Proximate nutrient composition (%DM basis) of basal feeds and pomegranate peel powder.

Proximate Nutrient(Per cent)	Broiler starter	Broiler finisher	Pomegranate (*Punica granatum*) peel powder
Dry Matter	90.58	90.62	91.60
Crude protein	22.48	20.25	7.61
Ether extract	3.49	3.90	1.28
Crude Fiber	4.73	4.26	19.39
Total ash	4.46	4.37	5.46
Nitrogen Free extract	64.84	67.22	66.26
ME (kcal/kg)	3055	3091	

Table 2. Ingredient composition of the diet

Ingredient	Starter	Finisher
Maize	58.0	62.2
Soybean meal	34.2	30.0
Oil	3.0	3.0
Stone grit	1.60	1.85
DCP	1.90	1.65
Salt	0.40	0.40
DL- Methionine	0.19	0.16
Lysine	0.14	0.12
Vitamin and mineral premix	0.5	0.5

Table 3. Composition of Multienzyme

Sr. No.	Enzyme	International Unit per Kg
1.	Amylase	33,42,000 IU
2.	Protease	12,25,000 HTU
3.	Cellulase	6,85,000 IU
4.	Beta-Glucanase	4,36,000 IU
5.	Xylanase	9,75,000 IU
6.	Pectinase	2,69,000 IU
7.	Phytase	1,40,000 FTU

Experimental starter feed, finisher feed and PPP were analyzed for proximate principles as per standard procedures (AOAC, 2005).

Performance Parameters

Daily feed intake and body weight were recorded, and weekly body weight gain was calculated. Feed conversion ratio, performance index, and protein efficiency ratio were also computed. After the feeding trial, a 5-day metabolism trial was conducted using one bird per replicate per treatment to evaluate dry matter digestibility and nitrogen retention. Nutrient metabolizability was assessed using the total collection method, with daily excreta collection. Feed and dried excreta samples were analyzed for proximate principles (AOAC, 2005), and nitrogen content was determined by the Kjeldahl method using an automatic nitrogen analyzer.

Carcass parameters

Broilers were humanely sacrificed following standard procedures (Panda, 1995), processed, and eviscerated for carcass evaluation, with individual weights of giblets recorded. Breast meat pH (Trout et al., 1992), water-holding capacity, and drip loss were measured using standard methods. Haematological parameters (haemoglobin, RBC, WBC, and PCV) were determined by conventional techniques, while serum biochemical parameters were analyzed using an Automatic Biochem Analyzer of Schiapparelli biosystems, INC, using standard kits.

Statistical analysis

The experimental data were subjected to statistical analysis (SPSS Ver. 24.0) using one way analysis of variance as described by Snedecor and Cochran (2004) to test for significant variation between treatment groups. Probabilities values of less than 0.05 ($P<0.05$) were considered significant. Comparison of mean values was carried out by Duncan's Multiple Range Test (Duncan, 1955). The results were interpreted and expressed as means ± pooled SEM.

RESULTS AND DISCUSSIONS

Growth performance

Cumulative feed consumption was significantly ($P < 0.05$) higher in the control group than in the treatment groups. These findings align with earlier reports showing significant effects of PPP supplementation on broiler feed intake (Hafeez et al., 2023; Ghosh et al., 2020) and increased feed intake with multienzyme supplementation (Attia et al., 2020).

Overall body weight gain was significantly

higher (P < 0.01) in broilers fed diets supplemented with PPP and multienzyme. These findings are consistent with earlier studies reporting improved body weight gain with PPP supplementation (Baqer and Ibrahim, 2022; Kamel et al., 2021), herbal additives combined with multienzymes (Singh et al., 2024; Tanwar et al., 2021), multienzyme alone (Attia et al., 2020), and herb–multienzyme combinations (Gaur, 2022).

The overall mean feed conversion ratio was found to be significant (P<0.05) on supplementation of PPP and multienzyme. The results of the present study are in agreement with Ahmed and Yang (2017) who observed improved (P<0.05) overall FCR of broiler supplemented with *Punica granatum* L. byproduct at level of 0.5% and 1% with basal feed. Similar findings were observed by Baqer and Ibrahim (2022) with 50 ml/literaquous extract of PPP.

Table 4. Effect of pomegranate (*Punica granatum*) peel powder and multienzyme on performance of broiler chickens

Attributes	Treatments				P value
	T1	T2	T3	T4	
Feed consumption (g/bird) (0-5 wk)	3017.08b±53.89	2772.00a±92.80	2837.00ab±37.97	2744.00a±81.94	<0.05
Body weight gain (g/b)	1550.62a±29.26	1632.30b±6.30	1762.30c±25.102	1715.86c±21.09	<0.01
Feed conversion ratio	1.95b±0.05	1.70a±0.06	1.61a ±0.027	1.60a±0.06	<0.05
Performance index	798.71a±35.28	964.57b±34.02	1095.41b±20.32	1077.64a±51.11	<0.01
Protein efficiency ratio	2.43a±0.06	2.72b±0.11	2.93b±0.02	2.90b±0.08	<0.01
Metabolizability of DM (%)	80.85a±0.22	82.46b±0.13	83.65c±0.05	83.45c±0.08	<0.01
Metabolizability of EE (%)	86.50a±0.41	86.91a±0.18	89.16b±0.20	88.72b±0.16	<0.01
Nitrogen retention (g/bird)	2.21±0.12	2.66±0.10	2.62±0.08	2.69±0.03	0.99

a, b, c- Means superscripted with different letters within a column differ significantly from each other.

** P<0.01, *P<0.05, NS= Non-significant

Table 5: Effect of pomegranate (*Punica granatum*) peel powder and multienzyme on offals weight (g) of broilers in different treatment groups

Attributes	Treatments				P value
	T1	T2	T3	T4	
Heart (g)	9.94±0.24	10.01±0.46	9.84±0.21	10.38±0.39	0.081
Liver (g)	36.24±0.58	37.35±1.31	35.57±1.01	34.84±0.06	0.075
Gizzard (g)	27.84±0.60	26.44±1.32	26.84±1.43	24.01±1.18	0.655
Giblet (g)	74.53±1.08	74.30±1.81	73.50±1.03	70.18±1.22	0.823
Drumstick (g)	162.75±4.785	166.50±4.941	179.25±3.816	171.00±7.188	0.688
Spleen (g)	1.80±0.039	1.84±0.090	1.75±0.043	1.81±0.183	0.105
Intestinal length (cm)	168.05±3.434	164.91±3.279	165.38±1.083	166.58±3.359	0.531

Table 6. Effect of pomegranate (*Punica granatum*) peel powder and multienzyme on drumstick weight (g), spleen weight (g) and intestine length (cm)

Attributes	Treatments				P value
	T1	T2	T3	T4	
pH	6.08±0.04	6.10±0.07	6.05±0.06	6.05±0.09	0.656
Water holding capacity (%)	32.51±0.28	36.14±2.20	36.42±1.05	32.49±0.75	0.068
Drip loss (breast) %	9.48b±0.22	8.00b±0.32	7.27a±0.78	8.20b±0.27	<0.05

Mean with different superscript differ significantly within a column

Significant (P<0.01) improved effect of supplementation of PPP and multienzyme was found on cumulative performance index in broiler chickens. The improved performance index observed with multienzyme supplementation agrees with Nizamuddin et al. (2013). Similar significant enhancements in performance index with combined herb and multienzyme supplementation have been reported by Sharma (2022), Gaur (2022), Chaudhary (2022) and Singh (2022).

Statistical analysis of the data revealed a highly significant effect (P<0.01) on the cumulative protein efficiency ratio, which was significantly higher in the treatment groups compared to the control group. Several studies have reported significant improvements in protein efficiency ratio (PER) with combined supplementation of herbs and multienzymes in broilers (Chaudhary, 2022; Singh, 2022; Sharma, 2022; Gaur, 2022). However, Gosai et al. (2023) reported a non-significant effect on overall PER with varying levels of PPP supplementation.

A highly significant (P<0.01) effect of PPP and multienzyme supplementation was observed on the metabolizability of dry matter, crude protein, and ether extract. Nitrogen balance was significantly higher (P < 0.01) in all treatment groups compared to the control. These findings are consistent with earlier reports showing improved nutrient digestibility and nitrogen utilization with herb and multienzyme supplementation (Chaudhary, 2022; Gaur, 2022; Sharma, 2022; Rezvani et al., 2018) and with multienzyme supplementation alone (Attia et al., 2020).

Supplementation of PPP and multienzyme showed no significant effect on dressed weight, eviscerated weight, organ weights, giblet weight, drumstick and spleen weight, or intestinal length of broilers, while a significant difference (P < 0.05) was observed in meat drip loss. The lowest drip loss was recorded in the T3 group. Similar observations have been reported by El-Rayes et al. (2023) and Singh (2022).

The statistical analysis of data showed no significant effect of supplementation of PPP and multienzymeon Hb (g/dl), RBC ($\times 10^6$/µl), WBC ($\times 10^3$/µl) and PCV (%).The results of the study are supported by Gosai et al. (2023) who observe non-significant (P>0.05) difference in Hb, RBC and WBC with supplementation of 0.25%, 0.5% and 1% levels of PPP on broilers. Contrary to this, Elnaggar et al. (2022) showed that Hb, RBC, WBC and PCV were significantly increased (P=0.001) with 0.25%, 0.5%, 1% and 1.5% levels of PPP supplementation on broilers. Similarly, Kamel et al. (2021) observed significant (P<0.01) results with supplementation of 0, 3, 6 and 9% level of PPP on Japanese quail.Regarding multienzyme, the results are in agreement with Mohammadigheisar et al. (2018) who recorded non-significant (P>0.05) effects on blood cells of chicken fed diet supplemented with 0.05% and 0.10% multienzyme.

All serum biochemical parameters remained within normal physiological ranges, and PPP and multienzyme supplementation showed no significant differences (P > 0.05) among treatment groups. Similar findings were reported by El-Rayes et al. (2023) for AST and ALT activities, and by Nizamuddin et al. (2013), Kumar et al. (2013), and Omojola et al. (2014) for enzyme supplementation, although El-Rayes et al. (2023) observed increased plasma protein fractions with higher PPP levels.

Table 7. Effect of pomegranate (*Punica granatum*) peel powder and multienzyme on haematological parameters of broilers in different treatment groups

Group	T1	T2	T3	T4
Hb (g/dl)	19.25±0.323	2.48±0.048	25.19±0.190	32.65±0.132
RBC (106/µl)	9.38±0.315	2.58±0.165	25.52±0.278	32.10±0.599
WBC (103/µl)	9.12±0.125	2.50±0.173	25.28±0.149	32.35±0.622
PCV (%)	9.75±0.144	2.62±0.131	25.15±0.155	32.30±0.178

Table 8. Effect of pomegranate (*Punica granatum*) peel powder and multienzyme on serum parameters at 35th day of age of experimental broilers in different treatment group

Group	T1	T2	T3	T4
Glucose (mg/dl)	215.49±0.41	215.95±0.33	215.96±0.63	216.26±0.99
Creatinine (mg/dl)	0.22±0.03	0.22±0.01	0.20±0.03	0.18±0.00
Albumin (g/dl)	1.43±0.13	1.20±0.05	1.22±0.05	1.44±0.44
Globulin (g/dl)	1.99±0.05	2.07±0.09	2.18±0.10	1.92±0.16
Total protein (g/dl)	3.42±0.07	3.27±0.09	3.39±0.05	3.36±0.09
Urea (mg/dl)	4.52±0.22	4.60±0.29	5.15±0.26	4.18±0.55S
GOT/AST (U/L)	253.02±1.61	255.92±0.89	253.00±1.43	255.40±1.02
SGPT/ALT (U/L)	17.88±0.14	17.98±0.26	17.15±0.45	17.62±0.33
Calcium (mg/dl)	8.73±0.90	9.52±0.37	9.45±0.23	9.00±0.14

CONCLUSION

From the finding of the present study, it can be concluded that supplementation with pomegranate (*Punica granatum*) peel powder @ 0.5% and multienzyme @ 0.05% alone and in combination causes improvement in live body weight, weight gain, feed conversion ratio and performance index as compared to that of control group of broilers. Thus, pomegranate (*Punica granatum*) peel powder and multienzyme in the rations of broiler chicks may be useful for the safe, economical and efficient production of broiler and this formulation of ration could be used as an alternative to commercial growth promoters.

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