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I Suresh Kumar Reddy
MVSc Scholar, Department of Animal Nutrition, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Ramesh Babu Potu
Assistant Professor and Head, Department of Animal Nutrition, College of Veterinary Science, Sri Venkateswara Veterinary University, Proddatur, Andhra Pradesh, India

S Venkateswarlu
Assistant Professor, Department of Poultry Science, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

E Tirupathi Reddy
Assistant Professor, Department of Poultry Science, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Corresponding Author:
Ramesh Babu Potu
Assistant Professor and Head, Department of Animal Nutrition, College of Veterinary Science, Sri Venkateswara Veterinary University, Proddatur, Andhra Pradesh, India

Effect of pomegranate peel powder (*Punica granatum* L.) and synbiotic on serum lipid profile and carcass characteristics of broilers

I Suresh Kumar Reddy, Ramesh Babu Potu, S Venkateswarlu and E Tirupathi Reddy

Abstract
To evaluate the effect of supplementation of pomegranate peel powder (*Punica granatum* L.) and synbiotic on serum lipid profile and carcass characteristics of broiler, 180 day-old chicks were randomly assigned to 6 dietary treatments with 3 replicates in each treatment and consists of 10 birds per replicate for 42 days. The diets prepared were basal/control diet – T_1; control diet supplemented with 200 mg/kg α tocopherol as positive control – T_2; control diet supplemented with pomegranate peel (PP) powder @ 4 g/kg feed – T_3; control diet supplemented with synbiotic (S) @ 0.5 g/kg feed –T_4; control diet supplemented with PP @ 2 g/ kg feed + S @ 0.25 g/ kg feed – T_5; control diet supplemented with PP @ 4 g/ kg feed + S @ 0.5 g/ kg feed – T_6. Serum cholesterol levels were significantly lower (p<0.05) in the groups fed with PP powder in the diets (T_3, T_5 and T_6) whereas triglycerides content was significantly lower in group fed with 4 g PP powder alone. The dressing % was highest (p<0.05) with T_4 group, while the abdominal fat content was lowest in T_5 group. In conclusion inclusion of PP powder and synbiotic improved the lipid profile in serum and improved dressing percentage of carcass and lowered abdominal fat in broilers.

Keywords: Carcass, lipid profile, pomegranate peel powder, synbiotic

Introduction
In commercial broiler production, efficiency of feed utilization may be improved by the addition of various feed additives such as antibiotics, antioxidants, anticoccidials, herbal preparations, enzymes, probiotics, acidifiers and organic acids (Kamal and Ragaa, 2014) [12]. u. Alpha trev Antibiotic growth promoters (AGP) have been used in the poultry industry at sub-therapeutic levels for decades to improve bird performance due to their low input cost and ease of incorporation into feed and water. However, currently consumers concern about possible antibiotic residues and resistance leading to restrictions of antibiotics use in poultry feed (Jang *et al*., 2007; Saberfar *et al*., 2008) [11, 19]. Thus, there is an immense pressure on poultry industry to phase out the use of AGP, which has stimulated increased interest in alternative natural growth promoters (Jang *et al*., 2007) [11]. Poultry nutritionists and researchers have tried other alternatives to address the issue associated with antibiotic residues and improve the broiler performance. Whatever be the mechanism of action of alternative to AGPs, the main characteristic of a good alternative of AGPs is, it must improve performance at least at an equivalent level as AGPs. Based on the proposed mechanism of action of AGPs, both microbiota modulating and immune-modulatory compounds could have potential to act as an alternatives to AGPs. There are many possible ways in which microbiota modulating compounds could influence the intestinal microbial population such as probiotics, prebiotics, synbiotics, phytobiotics, as an alternative to AGPs. The information available on the effect of phytobiotics, synbiotics and their combination on serum lipid profile and carcass characteristics in broilers is scarce. Hence, the present study was conducted to evaluate supplementation of PP powder and symbiotic on serum lipid profile and carcass characteristics in broilers.

Materials and Methods
Pomegranate peel powder and synbiotic
Pomegranate peels were procured from the local market. They are cleaned, cut into small pieces, dried at 40 °C for 2 days in hot air oven and powdered in willey's grinder for supplementing into broiler chicken diet.

The commercially available Synbiotic formulation Bio-Org+® consisting of prebiotic (Mannon oligosaccharides and Fructo oligosaccharide @ 6%) and probiotic mixture (*Bacillus*, *Lactobacillus* and *Enterococcus* @ 6 x 10^9/gm), was procured from M/S My Agri Nutrition, Kesariyur, Murungai (PO), Thottiyam, Tiruchirappalli, India.

Birds, management and diets

One hundred and eighty day old commercial (Cobb 400) straight run broiler chicks were obtained from a local hatchery near Chittoor town, Andhra Pradesh. On arrival, the broiler chicks were weighed individually and were randomly allocated to six treatment groups, each treatment contained three replicates with 10 birds per replicate. The experiment was carried out from day 0 to 42 days age of broilers. Isocaloric and isonitrogenous experimental diets were formulated for broiler pre-starter (0-14 days), starter (15-21 days) and finisher (22-42 days) phases as per the nutrient requirements of broilers (ICAR, 2013). The diet prepared were basal/control diet – T_1; control diet supplemented with 200 mg/kg α tocopherol as positive control – T_2; control diet supplemented with pomegranate peel (PP) powder @ 4 g/kg feed – T_3; control diet supplemented with synbiotic (S) @ 0.5 g/kg feed –T_4; control diet supplemented with PP @ 2 g/ kg feed + S @ 0.25 g/ kg feed – T_5; control diet supplemented with PP @ 4 g/ kg feed + S @ 0.5 g/ kg feed – T_6. All the chicks were housed in well ventilated, individual pens allocated to each replicate and reared in deep litter system with rice husk as litter material. Fresh and clean drinking water was made available at all the times. All the birds were vaccinated as per the standard vaccination schedule. Uniform managemental practices were followed for all the treatments. Two birds per replicate were slaughtered for estimating serum biochemical parameters and carcass characteristics.

Biochemical parameters

Carcass Characteristics

At the end of the experiment, two birds from each replicate and thus a total of six birds per each treatment were randomly chosen, weighed and slaughtered. Individual weights of eviscerated carcass were noted. The prime cuts (breast, drumstick and thigh) and giblets (liver, heart and gizzard) were collected, weighed and their percentages were calculated on carcass weight basis.

Statistical analysis

The data obtained in this experiment were subjected to one-way analysis of variance (Snedecor and Cochran, 1994) [21] and the differences between means were tested using Duncan's multiple range test with a significance at $P < 0.05$ (Duncan, 1955) [4]. All the statistical procedures were carried out using SPSS, version 22.0.

Results

Serum cholesterol levels were significantly lower ($p<0.05$) in the groups fed with PP powder in the diets (T_3, T_5 and T_6), whereas triglycerides showed significantly lower values in group fed with 4 g PP powder alone compared to other dietary groups.

Among carcass characteristics, only dressing percentage showed significant difference ($p<0.05$) and higher dressing percentage was observed in T_4 (73.87) and lower in T_1 (70.87), whereas similar in T_3, T_4, T_5 and T_6 (73.46, 73.87, 73.28 and 73.34), while T_5 (73.28) and T_6 (73.34) were correlating with control group (T_1), while T_2 (71.19) was intermediate and correlating with control group. But no significant differences were observed in the parameters like breast, thigh and visceral organs such as heart, liver, gizzard and giblets. There was a significant ($p<0.05$) difference in abdominal fat among treatment groups. Significantly higher abdominal fat was observed in control T_1, T_2 and T_6 (3.13, 2.69 and 2.55) and lower abdominal fat was observed in T_5 (1.63), T_3 and T_4 (1.89 and 1.84) while T_3 and T_4 were similar and not different from T_5 and T_6.

Table 1: Effect of supplementation of pomegranate peel powder and synbiotic on serum biochemical profile in broilers

Parameter	Treatments						
	T_1	T_2	T_3	T_4	T_5	T_6	P Value
Cholesterol (mg/dl) **	203.33^{a}±6.38	189.00^{a}±2.90	163.67^{b}±3.13	189.17^{a}±8.56	172.00^{b}±3.05	168.33^{b}±6.93	0.002
Triglycerides(mg/dl) **	124.33^{a}±1.45	121.17^{b}±0.70	118.33^{c}±0.95	122.83^{ab}±0.60	121.83^{ab}±0.79	121.17^{b}±0.30	0.001
ALT (U/L)	12.50±0.84	12.33±0.62	12.72±1.19	11.95±0.61	11.43±1.02	14.78±1.92	0.414
Uric acid (mg/dl)	4.53±0.23	4.75±0.24	4.92±0.73	4.67±0.17	4.02±0.06	4.15±0.18	0.411

^{abc}Values bearing different superscripts in a row differ significantly *($p<0.05$) **($p<0.01$)

Table 2: Effect of supplementation of pomegranate peel powder and synbiotic on carcass characteristics in broilers

Carcass trait	Treatments						
	T_1	T_2	T_3	T_4	T_5	T_6	P Value
Dressing %*	70.87^{c}±0.71	71.19^{bc}±0.5	73.46^{ab}±0.76	73.87^{a}±0.80	73.28^{abc}±1.26	73.34^{abc}±0.55	0.047
% of carcass weight							
Breast	28.35±1.15	31.52±0.71	31.17±0.71	30.77±1.08	30.96±1.015	30.72±0.69	0.209
Thigh	14.71±0.30	13.74±0.17	14.04±0.50	14.10±0.32	14.24±0.20	14.49±0.32	0.369
Heart	0.95±0.08	0.98±0.09	0.88±0.05	1.05±0.07	0.91±0.07	0.91±0.07	0.638
Liver	3.02±0.17	3.23±0.16	2.85±0.28	2.64±0.29	2.57±0.09	2.84±0.13	0.214
Gizzard	3.44±0.32	3.05±0.10	3.20±0.15	3.30±0.16	3.31±0.20	3.40±0.21	0.792
Giblets	7.40±0.52	7.27±0.19	6.94±0.40	7.00±0.48	6.79±0.21	7.14±0.25	0.856
Abdominal fat*	3.13^{a}±0.15	2.69^{a}±0.24	1.89^{bc}±0.47	1.84^{bc}±0.22	1.63^{c}±0.19	2.55^{ab}±0.13	0.002

^{abc} Values bearing different superscripts in a row differ significantly *($p<0.05$)

Discussion

The present study revealed that supplementation of PP powder had significant (p<0.05) effect on the serum cholesterol (mg/dl) and triglycerides (mg/ml) when compared to control. However, the group supplemented with 0.5 g of symbiotic S did not show any significance with the control (T_1) and group supplemented with vitamin E (T_2). Yaseen *et al*. (2015) [23] and Kishawy *et al*. (2019) [14] found significantly (p<0.05) decreased total serum cholesterol in the groups with pomegranate peel extract. In contrary to present study, Li *et al*. (2019) [16] reported significantly (p<0.05) lower triglyceride values when fed 1.5 g synbiotic per kg basal diet compared to control diet and the differences with present study may be due to variable culture and prebiotic combinations. The PP powder beneficially influenced the plasma lipid profile observed in present study. This may be through inhibiting the activities of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and sterol O-acyltransferase which are the two key enzymes responsible for cholesterol metabolism, reducing cholesterol absorption and increasing the cholesterol excretion (Esmaillzadeh *et al*., 2004) [5] and also PP powder may exert inhibitory effect on pancreatic lipase activity that inhibit fat absorption from intestinal tract and increased faecal excretion of fat (Lei *et al*., 2007) [15].

The results showed that supplementation of pomegranate peel powder and synbiotic had significant (p<0.05) effect on dressing percentage and abdominal fat whereas no significant difference was observed in breast, thigh, heart, liver, gizzard and giblet percentages. The dressing percentage was significantly (p<0.05) higher in T_4 compared to control. In accordance with present findings, Awad *et al*. (2009) [3] supplemented synbiotic @ 1 kg and 0.5 kg per tonne basal diet during starter and finisher phases respectively, and reported significantly higher dressing percentage compared to other dietary groups. Similarly, Hamady *et al*. (2015) [7] also reported significantly higher dressing per cent in groups supplemented with 10 g PPE per 100 kg basal diet when compared to control. Further, Hamad and Kareem (2019) [6] reported that supplementation of 1% and 1.5% PP powder in diet significantly improved dressing percentage compared to control. These may be due to the improved digestive function, and balanced microflora resulting from synbiotic supplementation because it could provide more available nutrients for muscle production (Abdel-Wareth *et al*., 2019 and Meng *et al*., 2010) [2, 17].

In accordance with our findings, Li *et al*. (2019) reported that incorporating Synbiotic @ 1.5 g per kg diet significantly (p<0.05) lowered absolute and relative abdominal fat. Similarly, Kishawy *et al*. (2019) [14] reported significant reduction in body fat by adding 0.5 g and 1 g of Pomegranate peel extract to the diet compared to control. Further, Yaseen *et al*. (2015) [23] found significantly (p<0.05) decreased abdominal fat in the groups fed with pomegranate peel extract @ 0.05 g and 0.1 g per kg compared to control. This beneficial effect may be due to pomegranate peel powder or extract had an inhibitory effect on lipid metabolism due to the hypocholesterolaemic and hypolipidemic effect, thus decreasing body fat precipitation (Kishawy *et al*., 2019, Hossin 2009 and Lei *et al*., 2007) [14, 8, 15]. In contrast to our findings, Sarangi *et al*. (2016) [22] and Kamel *et al*. (2021) [13] reported no significance in dressing percentage and abdominal fat by supplementing either PP powder or synbiotic.

The improved lipid profile observed in this study by supplementing 0.5 g Synbiotic in diet may be related with the microorganisms and oligosaccharides in the synbiotic, which deconjugate bile acids to produce free bile acids or dropping acetyl CoA carboxylase (as a limited enzyme in fatty acid synthesis) in the liver and adipose tissue (Ooi and Liong 2010 [18], Velasco *et al*. 2010 [22] and Li *et al*. 2019) [15].

Conclusion

The present study indicated that supplementation of PP powder and synbiotic and their combinations improved the lipid profile in serum and lowered abdominal fat content along with improved dressing percentage in broilers.

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