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Effect Of Using Pomegranate Peel Extract As Feed Additive On Performance, Serum Lipids And Immunity Of Broiler Chicks

Asmaa T Yaseen [1], Magdy El-Said H El-Kholy [1], Walaa M Abd El-Razik [2] and Mohamed H Soliman[3]

[1]Dept. of Nutrition and Clinical Nutrition, Faculty of Vet. Medicine, Zagazig University, Egypt

ABSTRACT

An experiment was conducted to study the effect of dietary supplementation with pomegranate peel (*Punica granatum* L) extract (PPE) on growth performance, serum lipids profile and immunity response as serum lysozyme content in broiler chickens. A total of 90, one day old Cobb broiler chicks were used. Birds were allotted into three experimental groups; each group allotted into 3 subgroups, 10 chicks /replicate. Group I was fed on basal diet, group II was fed on basal diet supplemented with 0.05 g PPE /kg of the diet and group III basal diet supplemented with 0.1 g PPE /kg of the diet. Body weight and feed intake were determined weekly. Body weight gain and FCR were calculated. After the end of the trial, 3 birds from each group were slaughtered for collection of the blood. The result of the showed no significant differences ($P < 0.05$) between all groups in body weight, weight gain, feed intake and FCR. Liver, heart and abdominal fat percent were significantly decreased ($P < 0.05$) in group II and group III. Total cholesterol and LDLP- cholesterol concentration in serum were significantly decreased ($P < 0.05$) in group II and group III, while HDL- cholesterol concentration were significantly increased ($P < 0.05$) in group II and group III, also triglycerides and VLDL-cholesterol were significantly decreased ($P < 0.05$) in group III only. Lysozyme content (µg / ml) was significant increased ($P < 0.05$) in group II and group III in compared to control group.

Key words: pomegranate, Performance, cholesterol, lysozyme and broiler

INTRODUCTION

Pomegranate peel, a waste product of the pomegranate industry with higher antioxidant levels than the juice itself, an attractive candidate as a nutritional supplement for animals feed. Pomegranate peel extract had high antioxidant capacity, considering the scavenging or preventive capacity against super oxide anion, hydroxyl and peroxyl radical. Pomegranate fruit peel exerted diverse pharmacological functions as antioxidant activity *(1,2)*. Feeding pomegranate extract (POMx) rich in poly phenols affect on performance, health, nutrient digestion, and immuno competence of calves in the first 70 d of age. Feeding POMx had no effect on intake or BW gain in the first 30 d of age, but after 30 d of age, both grain dry matter intake and BW gain decreased with increasing addition of POMx. Feeding POMx did not influence dry matter, organic matter, or starch digestibility, but it reduced crude protein and fat digestion *(3)*. PPE was reported as anti-obesity agent in a mouse model of high-fat diet induced obesity and hyper lipidemia, also PPE decrease serum total cholesterol (TC), tri glycerides (TG), glucose levels and TC/HDL-cholesterol, this may be due to inhibition of pancreatic lipase enzyme that will cause inhibition of intestinal fat absorption *(4)*. Pomegranate fruit rind powder (PGFRP) at the dose of 100 mg/kg orally as aqueous suspension was found to stimulate the cell-mediated and humeral components of the immune system in rabbits *(5)*.

MATERIALS AND METHODS

Ninety, one day old chicks of a commercial meat type (Cobb 500) obtained from a local hatchery were used in this study. On arrival they were weighed and randomly allocated to equal three treatment groups. Each contained three replicates and each replicate contained ten chicks. Birds were reared in a naturally ventilated open house with saw dust as litter and at a density 10 birds/m2. Continuous lighting and suitable temperature was provided throughout the experiment. All the chicks were vaccinated against Newcastle, avian influenza and Gamboro diseases. The basal diet was formulated according to the requirements *(6)* during starter period (0-21day in which the bird fed diet contained CP 23.04% and ME 3203.784 kcal/kg diet) and Grower- Finisher period (22-42 day in which the bird fed diet contained CP 20.1% and ME 3200.358 kcal/kg diet).

Table 1. Physical and chemical composition (%) of the experimental diets used in experimental stages

Feed ingredients	Experimental diets	
	Starter	Grower-Finisher
Yellow corn	57.2	63.5
Soybean meal, 48%	25.3	23.6
Corn gluten, 60%	6.5	3.5
Fish meal, 65%	3.75	2.5
Soybean oil	3.5	3.2
Calcium carbonate	1.2	1.2
Calciumdibasic phosphate	1.5	1.5
Common salt	0.3	0.3
Premix[1]	0.3	0.3
DL- Methionine, 98%	0.2	0.18
Lysine, Hcl, 78%	0.15	0.12
Toxenil	0.1	0.1

Table (2) show The experimental design

Group	Diet used
1	Basal standard diet without additives
2	Basal standard diet with PPE 0. 5 kg / ton diet
3	Basal standard diet with PPE 1 kg / ton diet

Growth performance parameters: Chicks were weighed weekly to determine body weight, also amount of feed consumption. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated.

Carcass traits: At the end of the experimental period three birds from each group were selected randomly, fasted over night, weighed then slaughtered by sharp knife to complete bleeding, followed by plucking the feather, evisceration and finally weighted to detect the dressing percentage. The dressed carcass weight, liver, gizzard, intestine, heart and spleen, bursa and abdominal fat were weighted and calculated as percent of life body weight.

Blood sampling, biochemical analysis and immunological parameter: samples were collected at age of 1 and 42 day. At 1 day samples were collected from slaughtering 5 birds selected randomly, samples collected into clean centrifuge tube without anticoagulant for separation of serum, the sera obtained were used for lysozymes to measure level of humeral innate immunity. At 42 day 3

birds per group selected randomly, samples were collected from slaughtering into clean centrifuge tube without anticoagulant for separation of serum; the sera obtained were used for lysozymes and biochemical analysis. Total cholesterol, triglycerides, HDL, LDL and VLDL were measured *(7)*. Lysozymes activity in the serum was measured by agarose gel cell lysis assay, the method described by *(8)*.

Preparation of pomegranate peels extract

Pomegranate peels were collected after removing peel from local pomegranate fruit. The peels were dried by hot air oven at 40 °C till the moisture content became about 8% (dry basis). The moisture content was determined by using oven drying at 105 °C until constant weight was achieved. The peels were grounded then used for extraction *(9)*.

Anti oxidants extraction Procedures

The extraction yield of antioxidant compounds from plant materials is influenced mainly by the conditions under which the process of liquid-solid extraction is carried out to separate a soluble fraction from a permeable solid.

The dried powder sample of pomegranate peel was extracted by maceration in ethanol 70% (polar solvent with a dielectric constant of 24). The solvent was added to the dried and ground peel in glass flask with glass cover at ratio 10:1 as begin with ratio 4:1 and the residue was re extracted 3-4 times until it is exhausted during maceration they were applied into a thermostatic water bath shaker at room temperature for 48hrs. The liquid extract was separated from solids by vacuum enhanced filtration through Whatman No. 1 filter paper. The alcohol was removed from the filtrate by using high-capacity evaporator (EYELA Rotary vacuum evaporator system). The ethanol free extract was dried by using lyophilizer. The dried extracts were collected and weighted to calculate the percent of extract yield *(9,10)*. The dried extracts were kept in dark at 4°c until used.

Statistical analyses: All the data were analyzed using the general linear model of *(11)*.

RESULTS AND DISCUSSION

Table 3. Effect of the dietary supplementation with pomegranate peel extract (PPE) on overall performance of broiler chicks (means ±SE)

Parameters	Experimental groups		
	Control	0.05% pomegranate +cont 1	0.1 % pomegranate + cont
Final body weight, g	2134.17 ± 81.86	2307.50 ± 67.84	2126.25 ± 78.66
Absolute weight gain, g	2088.50 ± 81.68	2261.17 ± 68.7	2079.25 ± 79.53
Total feed consumption, g	3290.12 ± 116.9	3360.67 ± 106.36	3126.24 ± 42.10
Overall FCR	1.58 ± 0.05	1.49 ± 0.002	1.51 ± 0.04

The obtained result showed that no significant differences (P<0.05) between groups fed 0.05% and 0.1% PPE and control one in final body weight, absolute weight gain, total feed consumption and feed conversion ratio, while increased percent of PPE decreased body weight, weight gain, FI and FCR non significantly(P<0.05).

These results were agreed with *(3)* Feeding pomegranate extract had no effect on

intake or BW gain in the first 30 d of age, but after 30 d of age, both grain dry matter intake and BW gain decreased with increasing addition of pomegranate extract to calves. Feeding pomegranate extract did not influence dry matter, organic matter, or starch digestibility.

Table 4. Effect of the dietary supplementation with pomegranate peel extract (PPE) on carcass traits of broiler chicks (means ±SE)

Parameters	Experimental groups		
	Control	0.05% pomegranate +cont	0.1 % pomegranate + cont
Dressing %	69.13 ± 1.18 [a]	68.60 ± 0.15 [a]	67.14 ± 0.46 [a]
Intestinal %	4.54 ± 0.31 [b]	4.89 ± 0.14 [ab]	5.81 ± 0.33 [a]
Liver %	2.79 ± 0.31 [a]	1.79 ± 0.03 [b]	1.81 ± 0.01 [b]
Gizzard %	1.91 ± 0.21 [a]	1.90 ± 0.05 [a]	1.93 ± 0.05 [a]
Heart %	0.39 ± 0.03 [a]	0.38 ± 0.01 [a]	0.31 ± 0.06 [b]
Spleen %	0.14 ± 0.03 [a]	0.13 ± 0.02 [a]	0.08 ± 0.004 [a]
Bursa %	0.13 ± 0.02 [ab]	0.17 ± 0.002 [a]	0.09 ± 0.01 [b]
Abdominal fat %	1.14 ± 0.029 [a]	0.76 ± 0.032 [b]	0.75 ± 0.027 [b]

The obtained result revealed significant decrease ($P < 0.05$) in liver, heart and abdominal fat percent between groups fed 0.05% and 0.1% PPE and control, while no significant differences ($P<0.05$) between them in dressing, gizzard and spleen percent.

The anti-obesity effect of PPE in a mouse model of high-fat diet induced obesity and hyper lipidemia *(4)*.

Table 5. Effect of the dietary supplementation with pomegranate peel extract (PPE) on lipid profile of broiler chicks (means ±SE)

Parameters	Experimental groups		
	Control	0.05% pomegranate +cont	0.1 % pomegranate + cont
Total cholesterol (mg/dl)	237.22 ± 13.99 [a]	168.20 ± 2.40 [b]	167.28 ± 4.11 [b]
TG (mg/dl)	92.93 ± 0.43 [a]	84.97 ± 1.01 [a]	73.38 ± 5.5 [b]
HDL-cholesterol (mg/dl)	57.08 ± 3.83 [b]	67.93 ± 1.07 [a]	69.42 ± 2.89 [a]
LDL-cholesterol (mg/dl)	198.72 ± 12.46 [a]	117.56 ± 2.78 [b]	112.54 ± 7.74 [b]
VLDL (mg/dl)	18.59 ± 0.09 [a]	16.99 ± 0.20 [a]	14.68 ± 1.10 [b]

Our results revealed that total cholesterol and LDLP- cholesterol concentration in serum were significantly decreased ($P < 0.05$) in groups fed 0.05% and 0.1% PPE, but HDL-cholesterol concentration were significantly increased ($P < 0.05$) in the same groups, while tri glycerides and VLDL- cholesterol were significantly decreased ($P < 0.05$) in group fed 0.1% PPE only in compared to control group.

PPE decrease serum total cholesterol (TC), tri glycerides (TG), glucose levels and TC/HDL-cholesterol, this may be due to inhibition of pancreatic lipase enzyme that will cause inhibition of intestinal fat absorption *(4)*.

Table 6. Effect of the dietary supplementation with pomegranate peel extract (PPE) on lysozyme content (microgram/ml) of broiler chicks (means ±SE)

Parameters	Experimental groups		
	Control	0.05% pomegranate +cont	0.1 % pomegranate + cont
lysozyme content (microgram/ml)	0.60 ± 0.05^{c}	0.86 ± 0.03^{b}	1.10 ± 0.05^{a}

The obtained result show that lysozyme content (µg / ml) was significant increased ($P < 0.05$) in groups fed 0.05% and 0.1% PPE in compared to control group.

Pomegranate fruit rind powder (PGFRP) at the dose of 100 mg/kg orally as aqueous suspension was found to stimulate the cell-mediated and humeral components of the immune system in rabbits *(5)*.

CONCLUSION

In conclusion, the supplementation of broiler diets with pomegranate peel extract decreased deposition of abdominal fat and decreased accumulation of fat in liver and around heart. Also it decreased total cholesterol, TG, VLDL, LDL-cholesterol concentration in broiler serum and improved bird immunity by increasing lysozyme content in broiler serum, but it not improved broilers body weight and weight gain, so PPE can be considered as anti obesity, hypo lipidemic and immunostimulant agent.

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الملخص العربي

تأثير استخدام مستخلص قشر الرمان كإضافات اعلاف علي الأداء ودهون الدم والمناعة في بداري التسمين

أسماء طه ياسين، مجدي السعيد حسن الخولي، ولاء محمود عبدالرازق ، محمد حسين سليمان
قسم التغذية والتغذية الاكلينيكية- كلية الطب البيطري- جامعة الزقازيق

تم إجراء هذة التجربة لدراسة تأثير إضافة مستخلص قشر الرمان كمضادات اكسدة طبيعية في بداري التسمين. استخدم في هذة الدراسة عدد ٩٠ كتكوت تسمين , قسمت إلي ثلاث مجموعات كل مجموعة قسمت الي ثلاث متكررات كل متكررة احتوت علي ١٠ طيور تم تغذيتهم علي ٣ علائق متساوية في الطاقة والبروتين لكل مرحلة عمرية.

المرحلة الاولي : عليقة البادئ (١-٢١ يوم) وتحتوي علي ٢٣,٠٤ % بروتين خام و ٣٢٠٣,٧٨ كيلو كالوري طاقة ممثلة لكل كجم عليقة.

المرحلة الثانية : عليقة النامي والناهي وتحتوي علي ٢٠,١ % بروتين خام و ٣٢٠٠,٣٦ كيلو كالوري طاقة ممثلة لكل كجم عليقة.

وكانت المجموعات كالاتي :

المجموعة الاولي : غذيت علي عليقة ضابطة بدون اضافات .

المجموعة الثانية : غذيت علي عليقة ضابطة مع إضافة ٠,٠٥% مستخلص قشر الرمان .

المجموعة الثالثة : غذيت علي عليقة ضابطة مع إضافة ٠,١% مستخلص قشر الرمان .

تم قياس وزن الجسم المكتسب ومعدل الوزن الزائد وكمية العلف المستهلكة ومعامل التحويل الغذائي كل اسبوع وفي نهاية التجربة تم اخذ عينات دم من ٣ طيور لكل مجموعة عن طريق الذبح لتحليل محتوي مصل الدم من الدهون والليزوزيمز .

اتضح من نتائج التجربة الاتي :

لا يوجد فروق معنوية بين المجموعات الثلاثة في كل من وزن الجسم المكتسب ومعدل الوزن الزائد وكمية العلف المستهلكة ومعامل التحويل الغذائي. ولكن وجد نقص معنوي في نسبة الكبد والقلب ودهون البطن وكذلك نسبة الكوليستيرول والدهون الثلاثية والدهون منخفضة الكثافة في المجموعات المضاف اليها مستخلص قشر الرمان مقارنة بالمجموعة ذات العليقة الضابطة . وايضا وجدت زيادة معنوية في نسبة الدهون علية الكثافة وكمية الليزوزيمز في المجموعات المضاف اليها مستخلص قشر الرمان مقارنة بالمجموعة ذات العليقة الضابطة .