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Title	Growth performance and immunity of broilers fed rancid oil diets that supplemented with pomegranate peel extract and sage oil
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Citation	Japanese Journal of Veterinary Research, 64(Supplement 2), S31-S38
Issue Date	2016-04
Doc URL	https://hdl.handle.net/2115/62029
Type	departmental bulletin paper
File Information	S31-38 Asmaa T. Kishawy.pdf



Growth performance and immunity of broilers fed rancid oil diets that supplemented with pomegranate peel extract and sage oil

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Abstract

The objective of this study was the assessment of the effect of pomegranate peel extract (PPE) and sage oil on reducing the damaging effect of rancid soybean oil on broiler performance, immunity and serum lipids. The experimental groups were, group 1: fed normal non-rancid soybean oil, group 2: fed rancid soybean oil, group 3: fed rancid oil and supplemented with 0.05% pomegranate peel extract (PPE), group 4: fed rancid oil and supplemented with 0.1% PPE, group 5: fed rancid oil and supplemented with 0.05% sage oil and gr. 6: fed rancid oil and supplemented with 0.1% sage oil. Inclusion of rancid oil in broiler feed had decreased ($p < 0.05$) final body weight, weight gain and feed intake (FI), while feed conversion ratio (FCR) had increased ($p < 0.05$) than other groups. Supplementation with PPE and sage oil had improved weight gain and FCR. Serum cholesterol, triacylglycerols (TAG) and abdominal fat had increased in group 2, but in groups 3, 4, 5 and 6 was decreased. Feeding rancid oil had deteriorative effect on broilers immunity parameters as lysozymes content, but PPE and sage oil supplementation had improved immunity parameters.

Key words: broiler performance, immunity, pomegranate peel extract, rancid oil and sage oil.

Introduction

Oils are used as highly caloric source in poultry ration, besides supplying energy, it improve utilization of fat soluble vitamins and increase diet palatability⁴. The presence of mono-unsaturated fatty acids and poly unsaturated fatty acids (PUFA) in oils that make it more susceptible to oxidation during storage or heating, that cause defect in fat and diet quality. Oil oxidation decrease palatability of ration due to rancid odor, also it has health risk on poultry due to formation of free radicals¹⁴. The deteriorative effect of free radicals not only concerned with reducing the nutrient utilization

of the diet by reacting with proteins, lipid and vitamins but also extended to be considered toxic to intestinal enterocytes³⁰. Rancid oils can cause growth depression for broiler by reducing FI that causes depression in weight gain and final body weight^{5, 23}.

Inclusion of oxidized oils in the ration accelerates the development of coronary heart diseases as atherosclerosis that is marked with an elevation in total white blood cells (WBCS) count and its subtypes²². The oxidized oil intake tends to elevate plasma level of total cholesterol, TAG low density lipoproteins (LDL-C) and very low density lipoproteins (VLDL-C) which are cholesterol

esters that lead to increase the incidence of atherosclerosis¹⁵⁾. Moreover the immune response of the body had decreased by inclusion of rancid oils in broiler ration through decreasing the jejunal concentration of secretory immunoglobulin and increasing serum endotoxin^{19, 20)}.

Lipid oxidation effect can be reduced with addition of natural or synthetic antioxidant to decrease nutrient damage and prevent free radicals action on the cells³⁰⁾. PPE had high antioxidant scavenging capacity against super oxide anion, hydroxyl and peroxy radical^{18, 31)}.

PPE was found to improve performance as body weight, weight gain and FCR by having anti-bacterial effect on pathogenic intestinal microorganism^{1, 11)}. PPE had suppressed lipase activity in the body causing decrease in abdominal fat pad, so consider as anti obesity agent, also PPE reduce level of serum lipids as cholesterol, TAG, LDL-C. And VLDL-C by inhibition of fat absorption from intestinal tract¹⁶⁾ and prevent the effect of rancid oils¹⁸⁾. PPE had stimulant effect on humeral, cell mediated immunity²⁷⁾ and enhance serum lysozymes activity¹³⁾.

Ethanollic extract of sage leaf (*Salvia officinalis*) and its essential oil have anti oxidant activity against free radical this activity is due to high content of total phenols, gallic acid, carnosic acid and carnosol compounds²⁶⁾. Sage oil was considered natural growth promoter as, it enhance body weight gain FI and FCR¹⁷⁾. The active substances as carnosic acid and carnosol present in sage oil had an inhibitory effect on serum TAG elevation and pancreatic lipase activity causing reduction in serum lipids as cholesterol, TAG, LDL-C and VLDL-C²⁾. The phagocytic activity which is one of immunity indicator had been elevated by addition of sage oil and oregano herbs to animal feeding, therefore sage oil can consider immune stimulant substance³²⁾.

Materials and methods

Three hundred, one day old chicks of a commercial meat type (COBB 500) were obtained from a local hatchery and were used in this study. On arrival they were weighed and randomly allocated to equal six treatments groups. Each containing five replicates and each replicate contain 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 was provided throughout the experiment. The starting temperature was 33°C then decreased gradually 2°C each week until reached 21°C at the 6th week. All the chicks were vaccinated against Newcastle, avian influenza and Gamboro diseases. Average body weight, body weight gain, feed intake and feed conversion ratio were measured weekly for each replicate.

The basal diets were formulated according to the requirements of NRC²⁵⁾. The diets were divided into two stages, starter stage extended from 0-3 weeks and grower finisher stage from 3-6 weeks (Table 1). The proximate chemical analysis for the used feed stuffs and the experimental diets for (moisture, crude protein (CP) and ether extract (EE)) were carried out according to AOAC³⁾.

Table1. Composition of experimental diets

	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
Dicalcium 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
<u>Calculated composition</u>		
ME, Kcal/Kg	3203.78	3200.358
CP, %	23.0446	20.099
EE, %	6.1438	5.9192
CF, %	2.4062	2.4217
Ca, %	1.1914	1.123
Available P %	0.4682	0.4394
Lysine, %	1.3020	1.1483
Methionin, %	0.6548	0.5575

¹Muvco premix: Each 2.5kg contain vit. A (10.000000 IU), vit. D3 (2.000000 IU), vit. E (10g), vit.k3 (1000mg), vit. B1 (1000 mg), vit. B2 (5g), vit. B6 (1.5g), pantothenic acid (10g), vit. B12 (10mg), niacin (30g), folic acid (1000mg), biotin (50 mg), Fe (30g), Mn (60g), Cu (4g), I (300mg), Co(100mg), Se (100mg) and Zn (50g)ME (metabolic energy), CF (crude fiber) and Ca (calcium)

Experimental groups were fed as following, group 1 was control group fed diet containing normal soybean oil without any additives, group 2 fed diet containing rancid soybean oil without additives, group 3 fed diet containing rancid soybean oil with addition of 0.05% of PPE, group 4 fed diet

containing rancid soybean oil with addition of 0.1% PPE, group 5 fed diet containing rancid soybean oil with addition of 0.05% sage oil and group 6 fed diet containing rancid soybean oil with addition of 0.1% sage oil.

To induce soybean oil rancidity, it was kept in oven at 80°C for 72hr in presence of oxygen¹²⁾. Then rancid oil and other oils in experiments were chemically analyzed for their chemical properties (Table 2) as refractive index (RI) according to Elsdon⁷⁾, free fatty acids (FFA) and acid value (AV) according to AOAC³⁾, iodine value (IV) according to Casimir⁶⁾ and peroxide value (PV) according to Gray¹⁰⁾.

Pomegranate peel extract was prepared from dried pomegranate peel of local pomegranate fruit according to Ma²¹⁾. Sage oil was obtained by oil pressing of dried grounded sage according to Rasmy *et al.*²⁶⁾.

At the end of the experimental period five birds from each group were selected, off food over night, weighed then slaughtered to collect blood from slaughter and to measure abdominal fat percent of life body weight.

Blood samples were collected at age of 1 and 42 day. At 1 day samples were collected from slaughtering 5 birds selected randomly, blood samples were 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 blood samples were collected for separation of serum, the sera obtained were used for lysozymes and biochemical analysis. Other blood samples were collected at 42 day on EDTA then used for WBCs counting.

Total serum cholesterol concentration was measured according to Finley *et al.*⁸⁾, Serum TAG concentration was determined calorimetrically according to Wahlefeld³³⁾, Serum HDL-C was determined calorimetrically according to Naito²⁴⁾, Serum LDL-C and VLDL-C was calculated according to Friedewald⁹⁾.

Lysozymes activity in the serum was measured by

agarose gel cell lysis assay, according to Schlitz²⁹). Data was subjected to one-way ANOVA using the General Linear Model Procedure of SAS²⁸). Means were compared using the Duncan test ($\alpha = 0.05$).

Results

The chemical analysis of normal soybean oil, rancid soybean and sage oil were shown in (Table 2). Presented data showed higher value of free fatty acids, acid value and peroxide in rancid oil compared to normal soybean oil, on the other hand Iodine value was lowered, while refractive index value not affected

Table2. Chemical properties of the normal soybean oil, rancid soybean oil and sage oil.

Parameters	Normal soybean oil	Rancid soybean oil	Sage oil
Refractive index (RI 60)	1.45913	1.45895	1.46087
Refractive index (RI 20)	1.473	1.47295	1.47487
Free fatty acids (FFA %)	0.19	0.21	6.31
Acid value (AV %)	0.37	0.41	12.55
Iodine value (IV %)	117.42	110.59	101.40
Peroxide value (meq/kg)	2.70	4.15	4.55

Feeding diets containing rancid oil and supplementation with PPE and sage oil on broilers performance are shown in (Table 3). The rancid oil-fed group showed significant decrease ($p < 0.05$) in final body weight and body weight gain than other groups, while 0.05%, 0.1%PPE and 0.1% sage oil had significant increased ($p < 0.05$) body weight and body weight gain than other groups. FI was significant decreased ($p < 0.05$) in rancid oil fed group than other groups, while it was significant increased ($p < 0.05$) in 0.1% sage supplemented group than other groups. FCR also

significant increased ($p < 0.05$) in rancid oil fed group than other groups.

Total leukocytic count, Heterocytes, lymphocytes and monocytes were significant increased ($p < 0.05$) in rancid oil fed group than other, while they significant decreased ($p < 0.05$) in 0.1% PPE supplemented group than other groups. Lysozymes also were significant decreased ($p < 0.05$) in rancid oil fed group, while it is significant increased ($p < 0.05$) in 0.1%PPE supplemented group and control group with normal soybean oil (Table 4).

Feeding broilers rancid oil had significant increased ($p < 0.05$) levels of total cholesterol, TAG, LDL-C and VLDL-C in serum than groups fed normal soybean oil, but supplementation with PEE and sage oil had improved their levels in the serum.

Table 3. Effect of dietary supplementation with PPE and sage oil on growth performance of broiler chicks (Means $\pm$ SE).

parameter s	normal soybean oil diet	rancid soybean oil diet	Pomegranate peel extract		Sage oil	
			0.05 %	0.1%	0.05%	0.1%
Initial body weight, g	45.60 $\pm$ 0.68	44.80 $\pm$ 0.73	45.20 $\pm$ 0.66	45.00 $\pm$ 0.95	44.80 $\pm$ 0.49	45.80 $\pm$ 0.73
Final body weight, g	1927.30 $\pm$ 22.33 ^b	1715.80 $\pm$ 29.84 ^c	2049.60 $\pm$ 8.65 ^a	2039.00 $\pm$ 12.67 ^a	1941.10 $\pm$ 11.18 ^b	2031.00 $\pm$ 6.93 ^a
absolute body gain, g	1881.70 $\pm$ 22.48 ^b	1671.00 $\pm$ 29.70 ^c	2004.40 $\pm$ 8.37 ^a	1994.00 $\pm$ 13.44 ^a	1896.30 $\pm$ 11.03 ^b	1985.20 $\pm$ 7.34 ^a
Total feed intake, g	3312.30 $\pm$ 65.47 ^{bc}	3103.50 $\pm$ 96.83 ^d	3396.60 $\pm$ 44. 48 ^{ab}	3454.40 $\pm$ 26.88 ^{ab}	3212.80 $\pm$ 20.94 ^{cd}	3549.60 $\pm$ 37.35 ^a
FCR	1.76 $\pm$ 0.04 ^b	1.86 $\pm$ 0.05 ^a	1.69 $\pm$ 0.05 ^b	1.73 $\pm$ 0.01 ^b	1.69 $\pm$ 0.01 ^b	1.79 $\pm$ 0.02 ^{ab}

Means in the same row carrying different superscripts are significantly different (P < 0.05)

Table 4. Effect of dietary supplementation with PPE and sage oil on total and differential leukocytic count (cell $\times$ 103 / μ l) and lysozymes content (microgram / ml) in broiler chicks (Means $\pm$ SE).

parameters	normal soybean oil diet	rancid soybean oil diet	Pomegranate peel extract		Sage oil	
			0.05 %	0.1%	0.05%	0.1%
Total WBCs	24.41 $\pm$ 0.16 ^c	31.67 $\pm$ 0.34 ^a	27.14 $\pm$ 0.15 ^b	24.99 $\pm$ 0.26 ^c	27.45 $\pm$ 0.30 ^b	26.84 $\pm$ 0.29 ^b
Heterocyt es lymphocyte s	4.83 $\pm$ 0.09 ^c	11.42 $\pm$ 0.31 ^a	7.04 $\pm$ 0.31 ^b	5.12 $\pm$ 0.13 ^c	7.69 $\pm$ 0.24 ^b	7.04 $\pm$ 0.22 ^b
monocytes	10.79 $\pm$ 0.20 ^c	18.76 $\pm$ 0.20 ^a	13.11 $\pm$ 0.19 ^b	11.22 $\pm$ 0.19 ^c	13.29 $\pm$ 0.19 ^b	13.09 $\pm$ 0.31 ^b
lysozymes	1.02 $\pm$ 0.03 ^d	2.08 $\pm$ 0.10 ^a	1.28 $\pm$ 0.07 ^{bc}	1.08 $\pm$ 0.02 ^{cd}	1.47 $\pm$ 0.11 ^b	1.19 $\pm$ 0.10 ^{cd}
	1.14 $\pm$ 0.05 ^a	0.76 $\pm$ 0.02 ^d	0.90 $\pm$ 0.02 ^{bc}	1.01 $\pm$ 0.05 ^b	0.87 $\pm$ 0.02 ^{cd}	0.96 $\pm$ 0.07 ^{bc}

HDL-C level significant decreased (p < 0.05) in rancid oil fed group, in the same time its level was significant increased (p < 0.05) in 0.1% PPE supplemented group than other groups. Abdominal

fat percent was significant increased (p < 0.05) in rancid oil fed group, while it was significant decreased (p < 0.05) in 0.1% PPE supplemented group than other fed groups (Table 5).

Table 5. Effect of dietary supplementation with pomegranate peel extract and sage oil on serum lipids (mg / dl) and abdominal fat % of life body weight in broiler chicks (Means $\pm$ SE).

parameters	normal soybean oil diet	rancid soybean oil diet	Pomegranate peel extract		Sage oil	
			0.05 %	0.1%	0.05%	0.1%
Total cholesterol	237.27 $\pm$ 1.95 ^c	268.65 $\pm$ 2.04 ^a	240.13 $\pm$ 2.53 ^c	228.37 $\pm$ 2.29 ^d	246.60 $\pm$ 1.66 ^b	239.17 $\pm$ 0.72 ^c
TAG	92.75 $\pm$ 0.26 ^{cd}	104.02 $\pm$ 0.48 ^a	93.78 $\pm$ 0.35 ^{bc}	89.50 $\pm$ 0.40 ^c	94.71 $\pm$ 0.46 ^b	91.87 $\pm$ 0.39 ^d
HDL-C	57.05 $\pm$ 0.63 ^b	45.01 $\pm$ 0.35 ^d	56.01 $\pm$ 0.54 ^b	64.69 $\pm$ 0.59 ^a	54.42 $\pm$ 0.44 ^c	55.75 $\pm$ 0.39 ^b
LDL-C	198.76 $\pm$ 1.45 ^c	244.45 $\pm$ 1.91 ^a	202.87 $\pm$ 2.79 ^c	181.58 $\pm$ 2.55 ^d	211.12 $\pm$ 1.67 ^b	201.80 $\pm$ 0.60 ^c
VLDL-C	18.55 $\pm$ 0.05 ^{cd}	20.80 $\pm$ 0.10 ^a	18.76 $\pm$ 0.07 ^{bc}	17.90 $\pm$ 0.08 ^c	18.94 $\pm$ 0.09 ^b	18.37 $\pm$ 0.08 ^d
Abdominal fat %	1.14 $\pm$ 0.01 ^e	1.96 $\pm$ 0.02 ^a	1.23 $\pm$ 0.03 ^d	0.81 $\pm$ 0.03 ^f	1.49 $\pm$ 0.03 ^b	1.39 $\pm$ 0.02 ^c

Means in the same row carrying different supers cripts are significantly different (P < 0.05)

Discussion

Rancid oils had harmful effect on the animal health due to the high content of free radicals that generated from oxidation of PUFA that causing an increase in the peroxide value and free fatty acids of the oil³⁰⁾. In our results, the depression in growth performance manifested in final body weight, body weight gain, FI and FCR in rancid oil fed group may explained as free radicals reduce the nutrient utilization by reacting with protein, lipid and vitamins^{5, 23)}.

Sage oil and PPE supplemented groups had improved growth performance which may be due to their antioxidants effect^{18, 26, 31)} that prevent toxic effect of free radicals on intestinal enterocytes and nutrient oxidation so, improve nutrient absorption and utilization^{17, 30)}. PPE also had anti-bacterial effect against pathogenic intestinal microorganisms which improve nutrient availability leading to an improvement in broiler performance^{1, 11)}.

Immunity of broiler chicks had decreased by inclusion of rancid oil in the diet as, total WBCs count and their subtypes had increased , while lysozymes activity was decreased that

may be explained as , toxic products of oxidized oils increase serum endotoxin and intestinal immunoglobulins^{19, 20)}. Oxidized oils also may accelerate the development of coronary heart disease which considered an inflammatory disease that causes increase in total WBCs count and their subtypes²²⁾. On the other hand PPE and sage oil supplemented groups had improved the humeral and cellular immunity of the broilers, which may be because of their radical scavenging capacity on plasma endotoxin that developed from oil rancidity^{19, 20, 32)}.

Total cholesterol and TAG levels in broiler serum had been elevated in group fed rancid oil in their diet that may be due to increasing cholesterol esters in blood circulation¹⁵⁾. High level of serum lipids may cause high lipids precipitation in lipid tissues especially abdominal fat. Supplementation with PPE and sage oil had caused depression in serum total cholesterol, TAG, LDL-C and VLDL-C-C in serum this may be explained as sage oil contain active substances as carnosic acid and carnosol and PPE had inhibitory effect on pancreatic lipase activity that inhibit fat absorption from intestinal tract that cause decrease fat precipitation in lipid tissues^{2, 16, 18)}.

In conclusion, PPE and sage oil can be used as antioxidant feed additive to reduce effect of oxidized oils and to improve growth performance, immunity and serum cholesterol in broiler chicks.

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