



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2018; 6(6): 1016-1019

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Received: 17-09-2018

Accepted: 18-10-2018

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Effect of dietary supplementation of ghee residue on fatty acid profile and serum bio-chemical parameters in Aseel native chicken

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Abstract

The biological experiment was designed to study the effect of feeding ghee residue on fatty acid composition of meat and serum biochemical parameters (total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein) in native chicken (Aseel) for a period of 12 weeks. A total of 300 day-old, unsexed, Aseel native chicken belonging to single hatch were utilized for this study. The treatment groups were fed with diets containing ghee residue at different inclusion levels, i.e. 0% (T1-control), 5% (T2), 10% (T3) and 15% (T4). The blood samples were collected from jugular vein of each group and separated serum were used for biochemical analysis. Total n-3 fatty acid profile of muscle samples (thigh, breast and skin) were not influenced by inclusion of ghee residue even up to 15 per cent in native chicken diets. Whereas, there was significant ($p < 0.05$) difference only in serum total cholesterol and non-significant difference was observed in saturated fatty acid, mono unsaturated fatty acid and poly unsaturated fatty acid. Hence, it is concluded that ghee residue could be included up to 5 per cent level in native chicken ration for improving the chemical composition of meat without affecting fatty acids profile and biochemical parameters.

Keywords: Aseel, ghee residue, fatty acid profile, biochemical parameters

Introduction

Poultry farmers are often facing a deep crisis due to an uncertain increase in the price of essential feed ingredients such as maize, fish meal, soya meal, vegetable oils, etc., thus resulting in severe erosion of the farmer's economic viability. There are many unconventional feed sources such as tamarind seed, azolla, acacia seed etc. that has been included in the ration to minimize the feed cost. Indigenous chicken are an important source of animal protein and could be very helpful in combating the nutritional deficiencies and generating income for the rural masses, especially in the developing countries like India. In recent years there is an increasing drift in consumer and farmer preference to native chicken. Although native chicken grow at a slower rate and produces less number of egg (75- 90) than improved commercial breeds, meat from native chicken is preferred by people because of its taste, leanness and pigmentation.

Ghee residue is one such alternate feed ingredient which is a brownish solid mass obtained as a by-product of ghee industry. About 33 per cent of total milk produced in India is being utilized for ghee preparation and the average yield of ghee residue is calculated as one tenth of ghee produced [4]. Ghee residue proved to be a good source of protein, energy and minerals, especially calcium and phosphorus. As ghee residue contains 32–70 per cent of fat, it is used as a potential substrate for enzyme production of lipase [9], used to improve the keeping quality of pet food in addition to lowering its cost [8] and also used as an alternative feed ingredient to formulate low cost fish feed [12]. It could be used as a potential alternate unconventional feed ingredient in native chicken rations. Ghee residue is available at a nominal cost throughout the year and it is being considered as a waste and can be effectively utilized in native chicken diet to reduce the cost of production as well to enhance the nutrient utilization. Though there are many reports on nutrient property of ghee residue and its utilization, its applicability as a feed ingredient to poultry is scanty. Hence, the present study was proposed to study the effect of feeding ghee residue on fatty acid composition of meat and serum biochemical parameters (total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein) in Aseel.

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Materials and Methods

A total of 300 day-old, unsexed, Aseel native chicken belonging to single hatch were wing banded, weighed and randomly allotted into four treatment groups. All the treatment had three replicates and each consisted of 25 chicks. The treatment groups were fed with diets containing ghee residue at different inclusion levels, i.e. 0% (T1-control), 5% (T2), 10% (T3) and 15% (T4), respectively and reared for a period of 12 weeks in deep litter system of management. All the chicken were reared under standard uniform management conditions. The ghee residue was included in the diet at graded levels to prepare experimental diets on *iso-caloric* and *isonitrogenous* basis. The native chickens (Aseel) were fed *ad lib.* with brooder and grower diets prepared at Central Feed Technology Unit, Kattuppakkam from 1 to 8 and 9 to 12 weeks of age, respectively. The ingredient and nutrient composition of the brooder and grower diets are presented in Table 1 and Table 2, respectively.

At the end of 12th week, blood was collected randomly from one male and one female of each replicate, totaling six per treatment. The collected blood was allowed to clot and centrifuged for 10 minutes at 2000 rpm to separate the serum. Serum samples were analyzed for total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein. The biochemical kits used for these assays were purchased from M/s. Agappe diagnostics. The recorded data subjected to statistical analysis [13].

Table 1: Native chicken brooder feed formula (0-8 weeks)

Feed Ingredients (%)	Control (T ₁)	GR 5% (T ₂)	GR 10 % (T ₃)	GR 15 % (T ₄)
Maize	61.06	52.48	41.05	30.00
Ghee residue	0.00	5.00	10.00	15.00
De-oiled rice bran	0.00	5.09	15.31	24.43
Soya	34.88	33.03	29.53	26.16
Calcite/LSP	1.00	0.87	0.80	0.64
DCP	0.50	1.00	0.75	1.22
Mineral Mixture Poultry	2.00	2.00	2.00	2.00
Methionine	0.01	0.01	0.01	0.01
Lysine	0.05	0.02	0.05	0.04
Salt	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Supplements (g)				
Vitamin AB ₂ D ₃ K ¹	10.00	10.00	10.00	10.00
B-complex vitamins ²	25.00	25.00	25.00	25.00
Trace minerals ³	50.00	50.00	50.00	50.00
Nutrient Composition				
Nutrient				
Crude Protein (%)	21.00	21.00	21.00	21.00
ME (Kcal/Kg)	2900	2900	2900	2900

1. One gram of Vitamin AB₂D₃K supplement contained 82500 IU of Vitamin-A, 50 mg of Vitamin-B₂, 12000 IU of Vitamin-D₃ and 10 mg of Vitamin-K.
2. One gram of B-Complex supplement contained 8 mg of Vitamin-B₁, 16 mg of Vitamin-B₆, 80 mg of Vitamin B₁₂, 80 mg of Vitamin-E, 120 mg of Niacin, 8 mg of Folic acid, 80 mg of Calcium pantothenate, 120 mg of Calcium and 300 mg of Phosphate.
3. One gram of Trace Minerals contained 54 mg of manganese, 52 mg of zinc, 20 mg of iron, 2 mg of iodine and 1 mg of cobalt.

Table 2: Native chicken grower mash feed formula (8 -12 weeks)

Feed ingredients (%)	Control (T ₁)	GR 5% (T ₂)	GR 10% (T ₃)	GR 15% (T ₄)
Maize	51.50	40.00	31.50	23.00
Ghee residue	0.00	5.00	10.00	15.00
De-oiled rice bran	22.80	33.00	39.00	45.80
Soya	21.60	18.20	15.60	12.50
Calcite/LSP	1.10	0.80	0.80	0.70
DCP	0.60	0.80	1.00	0.80
Mineral Mixture Poultry	2.00	2.00	2.00	2.00
Methionine	0.05	0.04	0.03	0.10
Lysine	0.00	0.00	0.00	0.00
Soda bicarbonate	0.05	0.05	0.05	0.05
Salt	0.25	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00
Supplements (g)				
Vitamin AB ₂ D ₃ K ¹	10.00	10.00	10.00	10.00
B-complex vitamins ²	25.00	25.00	25.00	25.00
Trace minerals ³	50.00	50.00	50.00	50.00
Nutrient Composition				
Nutrient				
Crude Protein (%)	17.50	17.50	17.50	17.50
ME (Kcal/Kg)	2700	2700	2700	2700

1. One gram of Vitamin AB₂D₃K supplement contained 82500 IU of Vitamin-A, 50 mg of Vitamin-B₂, 12000 IU of Vitamin-D₃ and 10 mg of Vitamin-K.
2. One gram of B-Complex supplement contained 8 mg of Vitamin-B₁, 16 mg of Vitamin-B₆, 80 mg of Vitamin B₁₂, 80 mg of Vitamin-E, 120 mg of Niacin, 8 mg of Folic acid, 80 mg of Calcium pantothenate, 120 mg of Calcium and 300 mg of Phosphate.
3. One gram of Trace Minerals contained 54 mg of manganese, 52 mg of zinc, 20 mg of iron, 2 mg of iodine and 1 mg of cobalt.

Estimation of serum total cholesterol

Serum total cholesterol was estimated [1] in which cholesterol esters are hydrolysed to cholesterol and fatty acids. The cholesterol is oxidized by cholesterol oxidase to form cholesten-3-one and H₂O₂. This H₂O₂ oxidizes 4-amino antipyrine and phenol to a red coloured compound, which can be measured in a double beam spectrophotometer at 505 nm.

Estimation of serum triglycerides

Triglycerides were estimated [2] in which triglycerides are hydrolysed by lipases to glycerol and free fatty acids. Glycerol is converted to H₂O₂ and dehydroxy acetone phosphate. H₂O₂ combines with 4-chlorophenol to form a pink coloured complex, whose absorbance is measured in a double beam spectrophotometer at 500 nm.

Estimation of high density lipoprotein

High density lipoprotein was estimated [10] in which 200 µl of serum and 200 µl of precipitating reagent (polyethylene glycol) was added in a test tube, then mixed well and incubated at 37^o C for 10 minutes. When centrifuged at 2000 rpm for 15 minutes the high density lipoprotein fraction remains in supernatant. Then high density lipoprotein is estimated as like serum total cholesterol.

Estimation of fatty acid composition in meat

Fatty acid composition was analyzed by the original method [3] with some modifications [5]. Lipids were extracted from

breast, thigh meat and skin samples (10 g) in 50 ml of Folch solution (chloroform: methanol is 2:1). KOH (0.88%) was added to this solution which is followed by vigorous mixing while capped and incubated at room temperature for 2 hours. Then, the upper layer was removed, and chloroform was evaporated using N₂ gas (99.999%). After cooling, 1 ml of methylating reagent (BF₃-methanol, Sigma Chemical Co., USA) was added to 100 µl of lipid, which was heated at 70 °C for 30 min. The samples were removed from the water bath and allowed to cool, after which 2 ml of hexane (HPLC grade) and 5 ml of distilled water were added to the samples. The samples were then vortexed and the upper layer removed. The fatty acid methyl ester dissolved in hexane was transferred to a GC vial.

Fatty acid composition was analyzed using a gas chromatograph (GC 6890N, Agilent, USA) equipped with a mass selective detector. A split inlet (split ratio, 50:1) was used to inject samples into a HP-5MS capillary column (30 m×0.25 mm×0.25 µm film thickness, Agilent, USA). The ramped oven temperature was 150°C for 3 min, increased to 180 °C at 2.5 °C/min and maintained for 5 min, and further increased to 220 °C at 2.5 °C/min and maintained for 25 min. Inlet temperature was 210 °C and the detector temperature 250 °C. Helium was the carrier gas at constant flow of 0.7 ml/min. The temperature of the mass spectrometer (MS) source, MS quadrupole, and transfer line into the MS were 230, 150, and 280 °C, respectively. The fatty acid composition was identified by a mass spectrum database (NIST Library, mass spectral search program, version 5.0, USA).

Result and Discussion

Fatty acid Composition of Meat

Fatty acid profile of muscle samples (thigh, breast and skin) collected from various treatment groups of native chicken i.e. control (without ghee residue) and experimental birds (rations containing 5 per cent, 10 per cent, 15 per cent levels of ghee

residue) is presented in Table 3. Statistical analysis of data on saturated fatty acid, mono unsaturated fatty acid, poly unsaturated fatty acid and total n-3 fatty acid revealed no significant difference between treatment groups. However a numerical increase of saturated fatty acid and decrease of unsaturated fatty acid was observed as the level of inclusion of ghee residue was increased at graded level.

The ratio of saturated fatty acid and unsaturated fatty acid of ghee residue was 65.5: 34.5. While feeding the ghee residue at different levels i.e., 5 per cent, 10 per cent, and 15 per cent, there was a non-significant increase of saturated fatty acid composition in meat of thigh, breast and skin corresponding to the increase in level of inclusion of ghee residue. The finding of this study revealed that the fatty acid composition of body tissues was modified by the composition of fatty acid of the experimental feed ingredient used in the experimental diet.

Serum Biochemical Parameters

The mean serum biochemical parameter of Aseel which was fed on graded levels of ghee residue is presented in Table 4. Statistical analysis of data on serum total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein revealed no significant difference between treatment groups except serum total cholesterol ($P \leq 0.05$) at 12 weeks of age.

The serum total cholesterol of T2 group recorded highest level (173.33 ± 3.2) among all the treatment groups and differ significantly with other groups. The result on serum triglyceride, high density lipoprotein, and low density lipoprotein revealed that there was no existence of significant difference between the treatment groups. However, T2 recorded highest numerical value of 83.82 ± 5.10 , 42.53 ± 2.92 in high density lipoprotein, low density lipoprotein respectively and T4 recorded the lowest level (69.95 ± 3.88 , 38.40 ± 2.05) among

Table 3: Effect of feeding ghee residue on (Mean ± S.E.) fatty acid composition of breast, thigh meat and skin of native chicken

Treatment group	Saturated fatty acids ^{NS}			Monounsaturated fatty acids ^{NS}			Polyunsaturated fatty acids ^{NS}			Total n-3 fatty acids ^{NS}		
	Breast meat	Thigh meat	Skin	Breast Meat	Thigh meat	Skin	Breast meat	Thigh meat	Skin	Breast meat	Thigh meat	Skin
T1 (Control)	36.1 ± 1.64	40.96 ± 1.78	43 ± 3.43	41.5 ± 2.27	38.73 ± 1.78	40.61 ± 3.45	19.4 ± 1.82	16.83 ± 1.88	12.5 ± 1.17	1.3 ± 0.20	2.07 ± 0.42	1.93 ± 0.44
T2 (GR-5%)	37.3 ± 1.74	41.56 ± 1.88	44.5 ± 3.13	40.14 ± 2.97	39.15 ± 1.71	40.28 ± 3.95	18.7 ± 1.79	15.88 ± 1.74	11.9 ± 1.07	1.1 ± 0.14	1.93 ± 0.44	1.72 ± 0.34
T3 (GR-10%)	38.4 ± 1.94	41.14 ± 1.99	45.4 ± 3.73	40.51 ± 2.37	38.73 ± 1.43	38.94 ± 3.05	17.8 ± 1.82	15.39 ± 1.62	12.2 ± 1.23	1.0 ± 0.16	1.65 ± 0.55	1.61 ± 0.51
T4 (GR-15%)	39.1 ± 2.04	42.83 ± 1.77	45.8 ± 3.03	39.7 ± 2.57	37.81 ± 1.59	38.7 ± 4.05	17.4 ± 1.52	15.29 ± 1.54	12.0 ± 1.04	1.1 ± 0.15	1.57 ± 0.47	1.51 ± 0.23

GR- Ghee Residue

NS – Non-significant

Table 4: Effect of feeding ghee residue on serum bio chemical parameters (Mean ± S.E.) of native chicken

Treatment	Serum total cholesterol* (mg %)	Serum triglycerides ^{NS} (Mg %)	Serum HDL cholesterol ^{NS} (mg %)	Serum LDL cholesterol ^{NS} (mg %)
T1 (Control)	155.17 ^a ± 4.80	70.67 ± 3.75	77.33 ± 2.24	37.88 ± 1.68
T2 (GR-5%)	173.33 ^b ± 3.2	70.17 ± 3.96	83.82 ± 5.10	42.53 ± 2.92
T3 (GR-10%)	159.33 ^a ± 4.84	68.83 ± 3.91	73.6 ± 4.01	38.92 ± 3.15
T4 (GR-15%)	157.17 ^a ± 2.52	66.83 ± 2.27	69.95 ± 3.88	38.40 ± 2.05

GR- Ghee Residue

NS – Non-significant

* - Significant at 5% level ($P \leq 0.05$)

treatment groups. The level of serum triglyceride is higher in T1 group and the level is getting decreased as the level of

inclusion of ghee residue increased.

The result showed that there exists no significant difference in

serum total cholesterol between the treatment groups except the birds receiving 5 per cent ghee residue. These birds recorded higher total cholesterol than control and other treatment groups. The result on level of triglyceride among the various treatment groups was found to decrease as the level of inclusion of ghee residue increased.

The above results are in disagreement with the earlier reports [7] who observed that serum total cholesterol of all the treatment groups did not differ significantly with control except the birds that received 15 per cent ghee residue which recorded higher total cholesterol than control. He also reported that the triglyceride level was found to decrease as the inclusion level of ghee residue was increased.

The cholesterol content increased with increasing levels of ghee residue in the diet. This present result of this study was in agreement with [6] who reported the same in broilers fed ghee residue at graded levels. The effect of dietary supplementation of ghee residue on the meat and egg of Japanese quails was evaluated [14] and they found that breast muscle cholesterol content was significant ($P < 0.05$) among the treatment groups and the highest level was observed at 15 per cent ghee residue supplemented group (92.86 mg/100 g) and lowest in control (70.63 mg/100 g). There is significant reduction in total cholesterol in 15 per cent ghee residue supplemented pigs compared to the levels in 0, 5 and 10 per cent ghee residue supplemented pigs [11], similar trend was noticed in the present experiment, a significant reduction in total cholesterol in 15 per cent ghee residue supplemented native chicken.

Conclusion

It is concluded that supplementation of ghee residue at graded level did not influence the fatty acid composition of meat and serum biochemical parameters. Meat cholesterol levels were also increased at this level but it doesn't exceed the normal levels for consumption. Similarly, ghee residue could be included at 10 per cent levels for improving total cholesterol without affecting the other biochemical parameters.

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