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Effect of Nonsaponifiable Fraction of Avocado Oil on Body Weight, Body Fat and Blood Lipid Profile of Broiler Chickens

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ABSTRACT

Accumulation of body fat is the major problem associated with initiation of various diseases in fast growing broiler chickens. Methods to restrict the accumulation of body fat have met with limited success. The objective of this study was to evaluate the effect of Avocado Non-Saponifiable Fraction (NSF) in broiler chicken (from day 14 for 27 days) on body fat accumulation, growth performance, organ weight and plasma lipid profile. Supplementation of avocado NSF for 27 days has resulted in positive changes in plasma lipid profile with 50% decrease of abdominal fat and 33.8% increase in body weight compared with normal control chickens. The Avocado NSF improved body composition in broilers through higher lean-to-fat ratio and hence has a potential to improve overall health profile and reduce metabolic diseases in chickens.

Key words: Avocado non-saponifiables, abdominal fat, broiler chicken

INTRODUCTION

The growth performance of broiler chickens has increased spectacularly over the last 30 years due to intensive selection for economic traits to obtain market weight of about 2.2-2.5 kg (Flock *et al.*, 2005) with 1.6-1.8 kg feed consumption per kg of growth (Havenstein *et al.*, 1994a, 2003a) within 42 days of rearing. Unfortunately, this fast growth is associated with several negative effects. Major problem of the modern broiler hybrids is their fat load (Havenstein *et al.*, 1994b, 2003b; Zhou *et al.*, 2006) along with many other disorders including sudden death syndrome (Bowes *et al.*, 1988), musculoskeletal disorders (Sanotra *et al.*, 2001; Lilburn, 1994), cardiovascular disease and liver damage (Hutchison and Riddell, 1990).

Currently, carcass weight of broilers includes more than 18% as fat in their carcass. Fast growth of broilers with high density rations increases the fat depots (Geraert *et al.*, 1996), especially in the visceral region and over 85% of this fat is not physiologically required for normal body function (Choct *et al.*, 2000). The abdominal fat weight is considered as a good predictor of the total body lipid (Delpech and Ricard, 1965) and its quantification provides reliable information about the carcass lipid in chickens (Ricard *et al.*, 1983). Since, the deposition of lipid in tissues is about 3-4 times more costly than deposition of the equivalent weight of muscle in terms of energy (Soller and Eitan, 1984); the fat accumulation is considered as an unfavourable trait for producers as a product with low economic value along with its negative effect on consumer acceptance (Emmerson, 1997). Consequently, this has pressurised the broiler chicken industry to reduce the fat content of its product (Jones and Ferrell, 1992) and there is a need to address this problem of excess fat accumulation in broilers.

At present, feed restriction (Fisher, 1984) and the inclusion of dietary additives that promote lean growth at the expense of fat deposition are the two commercially successful methods in use. According to the recent review by Fouad and El-Senousey (2014), both the methods have shown a decrease in body fat, but many reports have shown negative effect on body weight.

The Avocado (*Persea americana*) fruit has been appreciated and utilized by indigenous Meso-American people for at least 9000 years (Chanderbali *et al.*, 2008). Currently the avocado is cultivated in many parts of the world including India for its nutritious fruit. In addition to the fruit, the oil of avocado is also consumed for its health promoting effects. The NSF of avocado oil consists of unique functional micronutrients such as, phytosterols (Duester, 2001), furan lipids and fatty alcohols (Farines *et al.*, 1995) and has proven effective antioxidant on broilers (D'Silva and D'Souza, 2014). Hence, to evaluate the effect of avocado NSF supplement during growing and finishing period of broiler chickens on body fat, body weight and lipid profile, this study was undertaken.

MATERIALS AND METHODS

Commercially available refined oil of Avocado (Hass cultivar) was used for NSF extraction. Twenty four, 12 days old clinically healthy broiler chickens of COBB-400 strain with equal number of males and females (after the brooder stage) were selected from a flock of private poultry farm in Udupi, Karnataka. The feed was supplied by Godrej Agrovat Ltd., India as Crumbro Excel Starter and finisher feed. Enzymatic assay kits for glucose, cholesterol, HDL-C and triglyceride quantification were obtained from Agappe Diagnostics, India. All other chemicals and reagents of highest purity were purchased from commercial sources.

Nutritional value of crumbro excel broiler feed: Crumbro excel starter: Crumble type (for 11-18 days), Pellet type (19-27 days), to provide 3100 kcal kg⁻¹ metabolic energy with 21% protein and 4.5% fat; Crumbro Excel Finisher: Pellet type (28 day onwards), to provide 3200 kcal kg⁻¹ metabolic energy with 20.5% protein and 5% fat (Details of composition is available in website of Godrej agrovat Ltd., India).

Extraction of nonsaponifiable fraction of avocado oil: The NSF of avocado oil was prepared by the method of Kolhe *et al.* (1982) with modifications. The avocado oil (25 g) in ethanol/water (3:1 v/v, 100 mL) and KOH (17.5 g) was refluxed for 2-4 h, till the completion of saponification, followed by dilution with water and extraction of NSF with n-hexane. The hexane was removed using a rotary evaporator and the ethanol dissolved residue was collected and stored at 4°C till use.

In vivo studies on broiler chickens: This study was carried out according to the guidelines of Institutional Animal Ethics Committee, University of Mysore. The 12 days old (24 broiler) chickens were distributed into two groups having two replicates of 6 birds each (3 males and 3 females) in caged wire floor pens as control and experimental groups. A light/dark cycle of 18 h:6 h and a room temperature of 25-30°C were maintained throughout the experimental period. The birds were fed the Crumbro Excel Starter feed for 14 days, followed by finisher feed (Crumbro Excel finisher Pellets) for the last 14 days with water *ad libitum*. The NSF supplementation was initiated on the 15th day post hatching. The experimental group received 3 mg avocado NSF/hen mixed with small amount of feed through individual food bowl. The control hens were maintained similar to the test chickens except for the addition of NSF, throughout the experimental period (27 days).

Blood sampling: At every 10-12 day interval, the individual hen was fasted overnight (10 h), weighed to note the body weight and 2 mL of blood was drawn (by a trained technician) from the branchial vein into heparinized tubes for subsequent determination of glucose, total cholesterol, HDL-C, LDL-C, VLDL and triglyceride in plasma. At the end of 27 day experimental period (41 day of post hatching), the birds were individually weighed, then sacrificed and eviscerated. Subsequently, internal vital organs including liver, heart and fat pad from the proventriculus surrounding the gizzard, down to the cloaca were manually removed and individually weighed. Internal organ weights were calculated as percent of body weight. The tissues were stored in -40°C till the use.

Biochemical analysis of plasma: The plasma glucose (GOD-POD method), total cholesterol, HDL-Cholesterol (phosphotungstate method) and triglycerides were quantified according to the instruction manuals accompanying the diagnostic kits obtained from Agappe Diagnostics, India, using enzyme based colorimetric methods. The plasma VLDL-C was calculated by employing the Friedewald formula (Friedewald *et al.*, 1972) ($\text{VLDL-C} = \text{Triglycerides}/5$). The plasma LDL-C was calculated as the difference between total cholesterol and the sum of VLDL-C and HDL-C. All blood parameters were expressed as mg dL^{-1} of plasma.

Tissue lipid quantification: The tissue total lipid was extracted according to the method of Folch *et al.* (1957) and quantified by gravimetry. The tissue homogenate was prepared with chloroform-methanol mixture (2:1 v/v) maintaining 1 g/10 mL for liver and heart and 1 g/20 mL for adipose tissue, using a Potter-Elvehjem homogenizer. The homogenate was filtered through Whatman filter paper into a glass-stoppered vessel. The crude extract was mixed with 0.2 volumes of aqueous NaCl (0.58%) solution and the mixture was allowed to separate into two phases in a separating funnel. The lower phase was collected and the upper phase was rinsed three times with small volumes of ideal lower phase. All the organic layers were pooled and dried at 40°C in a rotary evaporator. Lipid content was calculated from the weight of the dried lipids (g of lipid/g of tissue).

The total cholesterol in tissue lipid was estimated by the method of Zlatkis *et al.* (1953). Briefly, 2.5 mL of solubilized lipid in ferric chloride-acetic acid reagent ($0.5 \text{ g L}^{-1} \text{ FeCl}_3 \cdot 6\text{H}_2\text{O}$ in acetic acid) was taken in a glass stoppered tube and 1.5 mL of concentrated sulphuric acid was added. The tube was stoppered and mixed and allowed to stand for 20 min. The OD was read against the blank at 560 nm in a spectrophotometer. For calibration, standard cholesterol (0.1 mg mL^{-1}) was processed similarly.

Preparation of liver homogenate: Liver tissue of 0.5 g was homogenized in 5 mL 0.1 M Tris-HCl buffer of pH 7.5 and centrifuged in a refrigerated centrifuge at $10,000 \times g$ for 30 min. The supernatant was used as enzyme source for various assays.

Estimation of aspartate amino transaminase (AST) and alanine amino transaminase (ALT) (Mohun and Cook, 1957): Buffered substrate for AST: 225 mM L-aspartate and 3.5 mM of 2-oxo glutamate was dissolved in 67 mM phosphate buffer and the pH was adjusted to 7.5. Buffered substrate for ALT: 560 mM of L-alanine and 1.4 mM 2-oxo glutarate was dissolved in 67 mM phosphate buffer and the pH was adjusted to 7.5.

To 1 mL buffered substrate, 0.05 mL of appropriately diluted homogenate was added as enzyme source, mixed and incubated at 37°C for 10 min. Then, 0.02 mL of aniline-citrate (4 M citrate in

water mixed with equal volume of aniline) was added. To the blank tube, 0.05 mL of homogenate was added after the addition of aniline-citrate. The tubes were further maintained for 10 min at 37°C and 1 mL of DNPH reagent (1 mM in 1 N HCl) was added. After 10 min, 10 mL of 0.4 N NaOH was added, vortexed and OD was measured at 520 nm. For ALT, same procedure was followed with ALT buffered substrate and the step involving aniline-citrate is omitted. Pyruvate (0.1 mM) was used as standard. Activity was expressed as μmoles of product/min/g liver.

Alkaline phosphatase assay (Modified method of Bowers *et al.*, 1977): The assay mixture included 0.1 M carbonate buffer, pH 10; 3.0 mM Mg^{2+} and 0.2 mM p-nitro phenyl phosphate (p-NPP), in a total volume of 1 mL. The reaction was initiated by the addition of the enzyme, allowed to occur at room temperature and OD was measured after 5 and 10 min incubation at 405 nm. Activity was expressed as μmole of product (p-nitro phenol)/min/g liver.

RESULTS

Both the control and test sets of the broiler chickens were maintained in identical conditions except the experimental chickens were supplemented with NSF. The weight of individual chickens were measured on the 0, 10th, 22nd and 27th day of experimental period and the percent weight change was calculated and recorded. Figure 1 shows the body weight gain in control and treated chickens. There was a linear increase in the body weight of both the control and NSF treated chickens (Fig. 1). However, the total weight gain in NSF treated set was significantly more than that of the control chicks ($p < 0.05$).

The effect of NSF supplementation on body weight and tissue weight of broilers is shown in Table 1. NSF supplemented test chickens showed significantly higher weight gain compared with control. There was no significant change either in absolute weight or in relative weight of liver and heart of the control and test set. Significant decrease was observed in abdominal fat pad of NSF supplemented chickens compared with control.

The enzymes of liver function in control and test chicken livers is shown in Table 2. There was no significant difference in the control and test group. The effect of NSF treatment on the blood parameters of control and test chickens is shown in Table 3. The plasma triglyceride (TG) in normal

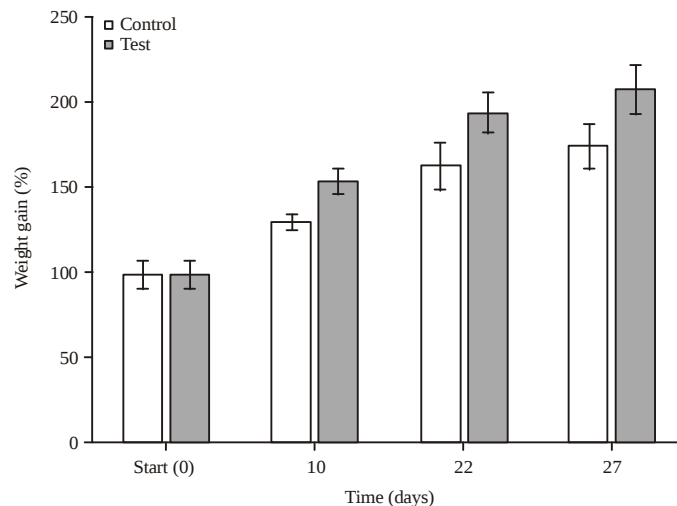


Fig. 1: Body weight gain in control and treated chickens

Table 1: Effect of NSF supplementation on body/tissue weight parameters

Body/tissue weight parameters	Normal control	NSF supplemented	p-value significance
Fold weight gain	1.76±0.26	2.10±0.29	0.046
Liver weight (g)	47.30±4.32	47.33±2.84	0.281
Heart weight (g)	9.34±1.29	9.97±2.13	0.457
Proventricular fat (g)*	25.46±7.75	12.73±1.38	0.009

Results are Mean±SD (n = 12), *Fat from the proventriculus surrounding the gizzard, down to the cloaca was weighed

Table 2: Effect of NSF on liver enzyme activity

Liver enzymes (U g ⁻¹)	Control	Test	p-value
ALP	0.346±0.127	0.302±0.013	0.255
ALT	42.910±0.36	42.880±0.68	0.436
AST	3.020±0.422	3.345±0.30	0.132

Activity of ALP, ALT and AST is expressed in μ moles of product released per minute U g⁻¹ of liver, Results are Mean±SD (n = 12)

Table 3: Effect of NSF on blood parameters of broilers at different weeks

Plasma parameters (mg dL ⁻¹) and sets of groups	Age of the chickens (days)			
	14	24	34	41
Triglycerides				
Control	74.48±11.3	102.20±28	149.74±30	143.25±42
Test	86.02±26.7	99.31±28.4	105.80±23.2	79.12±23.8
Total cholesterol				
Control	268.97±60	244.10±50.7	206.25±18.8	185.26±39.6
Test	264.41±49	240.16±54.5	212.49±36.02	174.89±26
HDL-C				
Control	117.29±28	120.70±24.6	81.91±25.7	76.04±26.4
Test	94.47±6.3	118.12±19.4	98.43±17.5	97.51±27.5
LDL-C				
Control	125.02±46	97.303±35	94.39±10.47	76.86±26.7
Test	130.34±23.5	102.18±32.5	76.12±23.8	62.74±8.8
VLDL				
Control	14.90±2	20.44±3.5	29.95±8	32.36±10
Test	17.20±5.3	19.86±5.7	21.16±4.7	14.64±4.8
Glucose				
Control	246.40±14.9	221.40±20.8	237.25±34.3	Not analyzed
Test	258.40±35	209.00±29.4	197.25±28.3	Not analyzed

Results are Mean±SD (n = 12)

control has shown a tendency to increase with age and increase was significant from 2nd to 4th week of experiment. On 27 days, 92.33% increase in plasma TG was observed in control set. In NSF supplemented chickens no such change in plasma TG was observed and remained constant as in the beginning of the experiment. The plasma total cholesterol and LDL-C concentration has shown age related decrease both in normal control and NSF supplemented chickens and no significant difference was noticed between the two sets. HDL-C had decreased significantly in normal control during the course of experimental period and no significant change in NSF fed chickens resulted in 22% higher HDL-C compared with control set. VLDL also showed age related increase in normal control with no significant change in NSF fed chickens.

The Fasting Blood Glucose (FBG) of normal control was maintained average 235 mg dL⁻¹ throughout the experimental period and the test chicken FBG decreased to average 203 mg dL⁻¹ on 10th day and maintained the same range thereafter.

The total tissue lipid and tissue cholesterol of heart, liver and abdominal fat (only tissue total lipid) are shown in Table 4. The heart and liver total lipid of NSF supplemented chickens decreased significantly compared to normal control. There was no significant change in total tissue cholesterol of heart but liver total cholesterol decreased significantly in NSF supplemented chickens compared with control.

Table 4: Effect of NSF supplementation on tissue lipid parameters of broilers

Table 1: Effect of NSF supplementation on tissue lipid parameters of broilers			
Lipids in various tissues	NSF supplementation hen day ⁻¹		p-value significance
	0 mg	3 mg	
Heart tissue (mg g⁻¹)			
Total lipid	58.93±15.9	41.13±8.1	0.007
Total cholesterol	5.85±1.62	5.39±1.24	0.334
Liver tissue (mg g⁻¹)			
Total lipid	74.80±19.5	53.30±11.04	0.051
Total cholesterol	10.23±1.76	5.52±1.35	0.004
Abdominal fat (mg g⁻¹)			
Total lipid	876.00±35	736.70±24	0.300

Results are Mean±SD (n = 12)

DISCUSSION

The NSF supplementation has shown significant improvement in growth rate of chicken and at the age of sacrifice, this set of chickens showed an average 33.8% higher body weight compared with control.

Biochemical evaluation of blood constituents in birds allow to monitor health, to detect subclinical disease (Bowes *et al.*, 1989) and to analyze metabolic alterations due to various endogenous and exogenous factors as in humans (Ross *et al.*, 1978; Bowes *et al.*, 1989; Meluzzi *et al.*, 1992; Krasnodebska-Depta and Koncicki, 2000; Harr, 2002; Jurani *et al.*, 2004; Rajman *et al.*, 2006). During the growth period, the blood lipid metabolites fluctuate in broilers strongly in association with energy metabolism. Various studies on different strains of broilers give a wide normal range for blood biochemical parameters. All the parameters studied during the course of our experiment were found to be within the normal range in both control and test chickens.

In broilers, HDL-C is the main fraction of serum total cholesterol (Hermier and Dillon, 1992; Peebles *et al.*, 1997). In Avocado NSF supplemented broilers, the HDL-C fraction had increased significantly ($p < 0.05$) with decrease in LDL-C compared with control. The plasma TG of control chickens has shown age-related changes with a tendency to increase significantly from 14th-35th day of post-hatch. Similar pattern of change in serum TG with age upto 7th week was observed by Meluzzi *et al.* (1992) and Peebles *et al.* (1997). The age related increase in plasma TG may be due to increased lipid percentage of finishing feed along with increased liver lipogenesis. The NSF supplementation inhibited this increase of plasma TG, suggesting effective lipid homeostasis in this set of chickens through decreased lipogenesis or increased energy utilization with increased muscle protein synthesis.

Deposition of fat in adipose tissue of birds depends directly on the TG rich plasma lipoprotein level (Hermier, 1997). Hence, reduction in plasma TG decreased the availability of TG for deposition in the adipose tissue and reduced the abdominal fat in broiler chickens with NSF supplement. There was no marked correlation for total plasma cholesterol with the total body fat but significant negative correlation was observed between plasma HDL-C and the body fat content. Similar correlation was reported by Gyenis *et al.* (2006).

Similar to blood lipid parameters, the Fasting Blood Glucose (FBG) of both test and control chickens were within the normal range although the FBG of test set was maintained at the lower range throughout the experimental course after initiating NSF supplementation. Typically the avian blood glucose concentration is reported as being between 190-220 mg dL⁻¹ (reviewed by Hazelwood (1986) and based on recent studies, the range is between 156-330 mg dL⁻¹ in young meat line chickens (Scanes, 2008).

In our study, at the age of 14th day, the blood glucose concentration ranged between 228-313 mg dL⁻¹. After 20 days of experimental period, at the age of 34 day, the blood glucose range was 165-225 mg dL⁻¹ in NSF supplemented chickens and 204-273 mg dL⁻¹ in the control set. The blood glucose concentration in broilers is known to be maintained within tight homeostatic mechanisms through several metabolic hormones such as insulin, glucagon, pancreatic polypeptide, corticosterone and thyroxin (Hazelwood, 1986). In view of the magnitude of variation in blood glucose in our study and according to the report of Scanes (2008), it is difficult to assume the existence of tight blood glucose homeostasis in broilers. The increased fat accumulation may result in insulin resistance and increased FBG in commercial broilers. The supplemented NSF probably through decreasing the adipose tissue, increases insulin sensitivity and thereby, decreases the FBG. Since, reduction of fat mass results in a significant decrease in lipid oxidation and enhance glucose homeostasis (Jazet *et al.*, 2008).

The lower blood glucose with increased body weight and decreased body fat in NSF supplemented broilers suggest possible increase of either insulin or insulin-like growth factor or their sensitivity by NSF. Since, these hormones increase protein synthesis and inhibit protein degradation in chickens (Duclos *et al.*, 1993) the observed growth promoting effects of NSF may be mediated through the action of these hormones.

In broiler chickens, about 80-85% of fatty acids that accumulate as triglycerides in adipose tissue are derived from blood lipids originated either from diet or synthesised in liver, the primary site of lipogenesis in birds (Saadoun and Leclercq, 1987; Hermier, 1997; Griffin *et al.*, 1992). The NSF supplementation in broilers has resulted in 50% decrease in abdominal fat pad, suggesting similar reduction in total carcass lipid (Summers *et al.*, 1992). Since, abdominal fat weight is a good predictor of the total carcass lipid (Delpech and Ricard, 1965), its accurate estimation predicts carcass lipid in broilers. The tissue lipid analysis confirms the same with a 30.2% decline in total lipid of heart and a 28.74% decline in liver total lipid as well as plasma TG compared with control.

Decrease in plasma TG, total tissue lipids and adipose tissue content in NSF supplemented broilers may be due to decreased liver lipogenesis or increased fatty acid oxidation by NSF. There was no significant macroscopic changes on both test and control chicken livers to indicate liver steatosis. Generally, increase in abdominal lipid accumulation, extends to liver steatosis resulting in marked macroscopic changes in liver including change in colour to pale yellow with swollen and dry texture (Tuncer *et al.*, 1987) with increased liver weight and focal haemorrhages (Shini and Bryden, 2009). The fat content of steatosis liver will be more than 30% of the liver (Shini and Bryden, 2009). Although the tissue lipid of the control group was higher than test group, it averages to 7.5% of liver, which is very less to show any macroscopic symptoms of steatosis.

The bioactive components of avocado NSF have shown decreased total lipid possibly through down regulating the PPAR- γ and thereby, decreasing the liver lipogenesis because whose transcriptional upregulation is responsible for liver lipogenesis in chickens (Richards *et al.*, 2010). Although no significant decrease in plasma cholesterol was observed in NSF supplemented broilers, total liver cholesterol was decreased significantly (46.04%). Cholesterol content of heart shown slight decrease (7.84%) due to very low cholesterol in that of control chickens.

Refined avocado oil was used for NSF extraction, which is known to have negligible amount of vitamin E due to its loss during the refining processes (Nicolosi and Orthoefer, 2004). The recommended allowance of vitamin E for effective hypolipidemic activity in broilers is 250-500 mg kg⁻¹ body weight (Sahin *et al.*, 2002) and the vitamin E content of fresh edible pulp of Avocado fruit (Hass) is 2.37 mg 100 g⁻¹ (USDA., 2011), which is insignificant compared to required

need of it. The feed enriched with vitamin E 5000 ppm and vitamin D₃ 0.6 MIU kg⁻¹ in starter/grower feed, 4000 ppm vitamin E and 0.56 MIU kg⁻¹ of vitamin D₃ in finisher feed was provided to both the control and the test hens during the experimental period. We claim that the hypolipidemic effect of NSF fraction may be due to various components other than vitamin E or vitamin D₃ because the Avocado NSF that was supplemented during the course of experiment was 3 mg chicken⁻¹.

The desirable increase in body weight through efficient utilization of food energy for the deposition of muscle weight with minimum fat accumulation was noticed during our study with broilers on NSF supplementation.

Improved body composition with higher lean to fat ratio is an important outcome of NSF supplementation in broiler chickens. The results are interesting both from an economic point of view and from the point of view of consumer's health. Studies have demonstrated that such higher lean-to-fat ratios improve overall health profile and reduce risk of heart attack, stroke and death from cardiovascular diseases in humans (Calling *et al.*, 2006).

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