ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 10 (4): 257-265, 2011
ISSN 1682-8356
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Effect of Njangsa Oil Seeds (*Ricinodendron heudelitii*) on Expression of Stearoyl-coenzyme a Desaturase in Laying Hens

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Abstract: Pathogenesis of liver steatosis in laying hen remains poorly understood. However, it has been suggested that regulation of the expression of Stearoyl-Coenzyme A Desaturase-1 (SCD-1) may alter hepatic lipid metabolism and prevent liver steatosis in laying hens. SCD-1 is an integral membrane protein that catalyzes the biosynthesis of monounsaturated fatty acids required for the synthesis of triglycerides, cholesterol esters and phospholipids. This study was conducted to determine whether Njangsa Oil Seed (NOS) (*Ricinodendron heudelotii*) which is rich in polyunsaturated fatty acids, specifically omega-3 fatty acids can regulate the expression of SCD-1 gene and lower hepatic lipid accumulation in laying hens. The experiment was carried out with seventy-two white leghorn hens (24 months old) which were randomly assigned to three layer diet supplemented with 0%, 10% or 20% NOS for 6 weeks. The mRNA levels in SCD-1 gene was quantified using forward and reverse primers specific to chicken SCD-1 in real-time reverse transcription-PCR (RT-PCR) technique with SYBER Green detection. Histological studies with liver tissues were performed with oil red stain and electron transmission microscope. Results indicate that NOS supplementation at 10% level tended to increase ($p>0.05$) SCD-1 mRNA expression in hens. Histological examination revealed micro-vesicular fat accumulation in the liver of hen fed NOS diets, indicating that supplementation of NOS in the diet may not prevent development of fatty liver. Results from this study suggest that NOS supplementation may regulate genes involve in lipid biosynthesis but may not prevent liver steatosis.

Key words: Njangsa oil seeds, stearoyl-coenzyme A desaturase-1, mRNA, laying hen, steatosis

INTRODUCTION

Fatty Liver Syndrome (FLS) is one of the most common metabolic disorders seen in laying hens in the period of high egg production. Liver cells distended with fat vacuoles and different size of hemorrhages (Riddell, 1997). Some studies have reported that the fat content of liver in the hen diagnosed with hepatic steatosis ranges from 40-70% dry weight (Riddell, 1997). Excess fat impairs liver functions and alters carbohydrate and protein metabolism and markedly impair glycogenesis and gluconeogenesis (Felig *et al*., 1970). Although the real cause of fatty liver disease in laying hens is not well understood, however excessive accumulation of lipids predominantly triacylglycerols in hepatocytes occur when VLDL transport is disrupted.

Triacylglycerol secretion in the form of VLDL from liver cells is highly dependent on the activity of stearoyl- CoA desaturase, which converts saturated fatty acids into monounsaturated fatty acids, specifically oleic acids (Cook, 1991). Hepatic packaging and secretion of VLDL require synthesis of apoB-100 as well as sufficient amount of oleic acid (C18:1, n-9), originating from a diet or synthesized by stearoyl-CoA desaturase (Ntambi, 1999). Recent studies with mouse model have reported that SCD1-deficient ($SCD1^{-/-}$) mice showed reduced synthesis of lipids, especially triglycerides (Miyazaki *et al*., 2001). $SCD1^{-/-}$ mice have low levels of triglycerides in VLDL and increased plasma HDL-cholesterol levels (Cohen *et al*., 2002; Huuskonen *et al*., 2001). These observations suggest that regulating SCD-1 gene would be a novel intervention strategy to improve poultry health. Thus, we hypothesized that incorporating njangsa oil seed (*Ricinodendron heudelotii)*, rich in omega-3 fatty acids in poultry ration would reduce hepatic triglyceride accumulation through its action on the expression of *SCD-1* genes. Njangsa (*Ricinodendron heudelotii*; family *Euphorbiaceae*), a tropical shrub from which oil seeds are harvested, grows or cultivated in West and Central Africa-specifically in Cameroon. *Ricinodendron heudelotii* reaches maturity between 4-5 years before producing fruits. The fruits are usually manually shelled to collect the seeds and dried. The seeds are grinded and used for soup and as an ingredient for seasoning baked meats and fish. The oil seed is an economical

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and valuable agricultural plant, especially in Cameroon. *Ricinodendron heudelotii* is a source of many nutrients and biologically active compounds that include omega-3 fatty acids, essential amino acids, minerals and antioxidant vitamins (Besong *et al*., 2010). The purpose of this study is to evaluate the effect of *Ricinodendron heudelitii* on expression of hepatic stearoyl-coenzyme A desaturase gene in laying hens which is involve in lipid biosynthesis.

MATERIALS AND METHODS

The feeding trial was conducted at the DSU Poultry Research Facility at the Hickory Hill farm. All procedures involving handling and treatment of birds were approved by the Animal Use and Care Committee at Delaware State University. At 24 months of age, seventy-two White Leghorn laying hens were placed in a conventional environment (16 h daylight:8 h darkness). Birds were weighed, paired and placed into wire mesh cages (2 birds/cage) with individual water-trough and feed-trough. Room temperature was maintained using heaters (Feature Comforts Flat Panel Electric Heater Model: CH-6B, Bionaire Tower Heater Fan Model: BFH3521-UM) and monitored from 29-45°C, using a digital thermometer (Acu-Rite 00611 Wireless Indoor/Outdoor Thermo-Hygrometer). A twelve hour light and dark cycle was provided using a digital timer (Intermatic Indoor Digital Wall Switch Timer - EJ500C). Prior to the treatments, birds were fed a standard layer diet without njangsa (NOS) for two weeks so as to acclimatize the birds to a new environment. At the end of the acclimatization period, birds were weighed and randomly assigned to three layer diets supplemented with 0% NOS (control); 10% NOS; 20% NOS for 60 days. Njangsa oil seeds obtained from Cameroon, Africa were and stored at 4°C, blended and mixed into the feed with the aid of a feed mixer. Experimental diets and drinking water were provided *ad libitum* for 60 days. The experiment contained three replications with eight birds per replicate. Experimental diets (Table 1) were formulated to meet nutrient requirements recommended by the National Research Council (1994).

Sample collection: Approximately 100 g sample of each of the experimental diets was collected and stored at -20°C for later analysis of fatty acids profile, protein and amino acids. Bird weights were recorded at the beginning of the feeding trial and bi-weekly during experimental period. At the end of the experimental period (day-60), four birds per replicate (12 birds per treatment) were sacrificed by cervical dislocation, liver was harvested and weighed. Approximately 5 gram sample was collected from each bird and immediately frozen in liquid nitrogen in pre-labeled fifty milliliters tubes and stored at -80°C for RNA extraction and SCD-1 mRNA quantification. Additional liver samples were collected and placed in a ninety-nine percent formaldehyde preservative solution and stored at -20°C for histological examination of lipid droplets.

Table 1: Composition of experimental layer diets

Ingredients	0% NOS	10% NOS	20% NOS
Composition by calculation (%)			
Corn (CP, 8.5%)	58.00	58.50	53.00
Soybean (CP, 43%)	23.00	22.50	18.00
Alfalfa	10.00	0.00	0.00
Njangsa Oil Seed (NOS)	0.00	10.00	20.00
Dicalcium phosphate	0.60	0.60	0.60
Limestone	7.59	7.60	7.60
Salt	0.25	0.25	0.25
Vitamin promix[1]	0.25	0.25	0.25
DL methionine (99%)	0.05	0.05	0.05
Grease	0.26	0.25	0.25

[1]Supplied per kilogram of diet: Vitamin A (retinyl acetate), 8,090 IU; cholecalciferol, 1,575 IU; dl-α-tocopheryl acetate, 12 IU; vitamin B12, 16 μg; vitamin K (menadione sodium bisulfate) 2.0 mg; riboflavin, 4.0 mg; pantothenic acid, 12.8 mg; niacin, 75 mg; choline, 509mg; folic acid 1.62mg; biotin 75μg; ethoxyquin 15mg

Lipid extraction: Total fat was extracted from experimental diets as described by Folch *et al.* (1957). Approximately 1 g of feed sample was homogenized for two minutes in chloroform/methanol mixture (2:1) to a final volume of 20 milliliters. The mixture was incubated for two hours on an orbital shaker in Para film sealed flat bottom flasks. The homogenate was centrifuged at 4000 rpm for five minutes and the supernatant siphoned into 50 ml conical tubes. Approximately 4 ml of water was added, vortexed and centrifuged at low speed to separate the two phases. The lower bottom phase containing the total fat was rinsed twice with methanol/water (1:1), dried and stored at -20°C for fatty acid analysis using gas chromatography.

Lipid analysis: The lipid profiles of the experimental diets were determined by gas chromatography. Total fat from each sample was vacuum dried for chromatographic analysis. Prior to GC analysis, samples were resuspended in 10.0 ml of dichloromethane (CH_2Cl_2), thoroughly mixed and centrifuged. A 20.0 μl of the supernatant was transferred to a sample vial and 980.0 μl dichloromethane added, capped and vortexed. A final dilution of 20.0 μl solution and 980 μl dichloromethane was made. For each sample, approximately 500 μl of dichloromethane phase of each sample was transferred to gas chromatographic vials and sealed. Fatty acids were analyzed as methyl esters, using capillary gas chromatography. The 37 component Fatty Acid Methyl Ester (FAME) standard and a SUPELCO SP-2560 0.25 mm x 0.25 μm x 100 m column for separation was used to confirm the identity of key fatty acids in the feed samples. The Shimadzu GC-2014 with a Flame Ionization Detector (FID), an AOC-20i

auto sampler was use to analyze all samples. Inlet temperature was set at 250°C, while the column temperature was set at 140°C for five minutes, then increased to 250°C at 4°C/minute and held at 250°C for 10 min. Helium was used as carrier gas, while nitrogen was used as make-up gas.

Histology of lipids in the liver tissue

Oil red stain: A 30 ml of Oil red O (Sigma) stock solution was diluted with 20 ml distilled water and incubated at room temperature for 10 min and then filtered into coplin jar. Approximately, 8 μm thick sections were fixed in Para formaldehyde for one hour followed by rinsing in distilled water. Samples were dehydrated with 50% ethanol and stained-with Oil Red O saturated in 70% ethanol for one hour. The sections were rinsed in 50% ethanol and then in tap water. Samples were counter stained with Harris hematoxylin for 1 min followed by another rinsing in tap water and then mounted in glycerin jelly. Stained liver samples were observed with a light microscope.

Transmission Electron Microscope (TEM): To observe lipid droplets in liver tissues with transmission electron microscope, tissue samples were prepared by placing 1 mm^3 section of liver samples in 0.1 M Sodium Cacodylate (SC) buffer of pH 7.4 and later rinsed twice in 0.1 M SC and then fixed in 2% glutaraldehyde and 2% para formaldehyde for one hour on a rotator. Tissues were washed three times in 0.1 M SC, fixed for two hours in osmium tetroxide, followed by two washes in 0.1 M SC, then twice in deionized water. Dehydration of samples was performed in 25, 50, 75 and 90% ethanol and held overnight. Tissues were dehydrated twice in 100% anhydrous ethanol and n-butyl glycidyl ether (n-BGE) for 30 min, then dehydrated in 100% n-BGE. Samples were infiltrated with a resin for one hour on a rotator followed by overnight infiltration in 100% Quetol (1 part n-BGE, 1 part Quetol). After embedding, samples were sectioned using ultra microtome to 60 nm thick, flattened with heated filament, collected onto 200-mesh copper (TEM) grids and stained. Grids were allowed to dry with heat before imaging.

Total RNA preparation and determination of mRNA SCD-1: Total RNA was extracted from 100 mg liver tissues using the TRI Reagent (Sigma) according to the manufacturer's instructions. Integrity of RNA and beta-action was confirmed on a 1.2% denaturing agarose gel stained with ethidium bromide (Fig. 1). Total RNA (100 ng/μl) was treated with RNase-free DNase 1 (Invitrogen) and reverse transcribed using forward and reverse primers specific to chicken stearoyl-CoA desaturase (Table 2). Beta-action (housekeeping gene, gene bank accession No. L08165) was reverse transcribed using forward and reverse primers specific to chicken beta-action (Table 2).

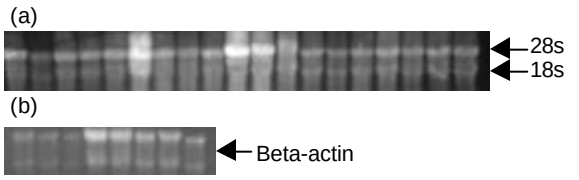

Fig. 1: Integrity of total RNA (a) and beta-action (b)

Table 2: Primer sequence of stearoyl-coenzyme A desaturase-1 (SCD-1) and β-action (house-keeping gene)

Primer	Primer Sequence	Length
SCD-1 F[ac]	CCACCATACATTCCCTACG	250 bp
SCD-1 R[bc]	CGCTCTTGTGACTCCCATCT	250 bp
β-action F[d]	GCACCACACTTTCTACAATG	250 bp
β-action R	ACGACCAGAGGCATACCAG	250 bp

[a]Forward. [b]Reverse.
[c]Genebank accession number for SCD-1 = X60465
[d]Genebank accession number for β-action = L08165

Messenger RNA quantification analysis by real-time PCR: Real-time PCR was carried out using Sigma SYBR Green RT-PCR kit (Sigma Chemical Co., St. Louis, Mo.). PCR amplification of *SCD-1* gene and *beta-action* gene conditions were 44°C for 30 min and 40 cycles of 95°C for 1 min, 58.8°C for 1 min, 72°C for 1 min and an optical read step at 80°C for 5 sec. Two negative controls (one with no template and the second with no transcriptase) were included. Cycler was programmed to run a first derivative melt curve after the final PCR step to check for false positives and primer-dimers. A default Cepheid Smart Cycler optics graphs threshold of 30 units was assigned.

Optics graphs showing the fluorescence intensity of each reaction plotted against PCR cycles were obtained for all samples for each run using the software provided with the cycler. A default Cepheid Smart Cycler optics graphs threshold of 30 units was assigned. Samples showing high and low Cycle threshold (Ct) values were recorded. Melt graphs and first-derivative melt graphs were obtained immediately after the completion of the PCR using the software and graphic program provided with the Cycler. The sample with lowest Ct value was used to determine a standard curve as described by Richards *et al.* (2004a,b). Ct values from the real time PCR was used to plot a standard curve by assigning a value of 1 RT-PCR unit (RT-PCRU) to the highest dilution showing a positive Ct and 10, 100 and 1,000, 10,000 RT-PCRU to the lower dilutions. The relative expression of SCD-1 gene was analyzed as described by Richards *et al.* (2004a,b) using beta-action gene for normalization.

Statistical analysis: Data from all treatment groups were analyzed using one-way ANOVA of SAS (SAS Institute, 2000). Differences among treatment means were determined using a Two-Sample t-test. Statements of statistical significant differences were based on $p<0.05$. Results were presented as means±SD.

Table 3: Fatty acids[a] profile of njangsa oil seed and experimental diets

	% of total fatty acids			
Fatty acids	NOS[b]	0% NOS	10% NOS	20% NOS
Myristic	0.37	0.44	0.13	0.07
Myristoledic	0.14	N.D[c]	0.03	0.08
Palmitic	18.90	14.70	8.93	7.46
Palmitelaidic	0.07	0.08	0.05	0.03
Palmitoleic	0.16	1.01	0.33	0.13
Heptadecanoic	0.35	0.13	0.12	0.09
10-Hepadecanoic	0.19	0.07	0.06	0.03
Stearic	15.20	3.12	4.60	5.89
Oleic	17.30	28.10	15.40	11.00
Linoleic	23.20	47.00	36.40	32.80
Alpha linolenic	0.13	2.70	0.89	0.56
Arachidic	0.45	0.42	0.26	0.21
11-Eicosenoic	0.49	0.44	0.34	0.32
Eicosapentaenoic	19.60	0.42	31.60	40.80
Docosadienoic	0.45	0.12	0.05	0.02
Docosapentaenoic	0.52	N.D	0.06	0.08
Docosahexaenoic	0.51	N.D	0.05	0.06
Tricosanoic	0.26	0.09	0.07	0.06
Lignoceric	0.30	0.25	0.14	0.09
Nervonic	0.45	N.D	0.04	0.06
Total fat per serving	32.80	2.13	6.82	10.70
Saturated fat, g/100 g	12.10	0.42	0.99	1.50
Saturated fatty acids % of fat	36.70	19.70	14.60	14.00
Polyunsaturated fats (Total) g/100 g	14.60	1.08	4.71	7.96
Poly-unsaturated fatty acids % of fat	44.40	50.50	69.10	74.40
Monounsaturated fats (Total) g/100 g	6.08	0.63	1.11	1.23
Mono-unsaturated fatty acids % of fat	18.50	29.70	16.20	11.50
Omega 3 fatty acids (Total) g/100 g	6.81	0.07	2.23	4.44
Omega 3 fatty acids (Total) % of fat	20.76	3.13	32.64	41.49
Omega 6 fatty acids (Total) g/100 g	7.74	1.01	2.49	3.51
Omega 6 fatty acids (Total) % of fat	23.60	47.37	36.49	32.84
Omega 9 fatty acids (Total) g/100 g	5.66	0.60	1.05	1.17
Omega 9 fatty acids % of fat	17.25	28.13	15.43	10.98
Analysis[d]				
Crude protein (%)		17.54	16.42	16.42
Total calories (cal/g)		3,640	3,833	3,845

[a]Results expressed as a percentage of the total fatty acids.
[b]Njangsa Oil Seed (NOS).
[c]Not Detectable (N.D).
[d]Analyzed by Midwest Laboratory, Inc., Omaha, Nebraska

RESULTS

Nutrient analysis of the experimental diets revealed that Njangsa Oil Seeds (NOS) contain: 44.4% polyunsaturated fatty acids, 19.6% eicosapentaenoic acid (EPA, C20:5) and 20.76% omega-3 fatty acids (Table 3) and 24.7% crude protein and all essential amino acids (Table 4). The content of omega-3 fatty acids appear to be higher ($p>0.05$) in layer diet supplemented with NOS compared to control diet (Table 3).

Body weight of birds from all treatment groups was not different ($p>0.05$) throughout the experimental period, thus, indicating that incorporation of NOS in the diet did not have an effect on body weight (Fig. 2). In addition, we also observed that liver weight was not significantly ($p>0.05$) different from birds in all treatment groups. However, the liver weight of birds fed 20% NOS diet tended to be greater ($p>0.05$) than those fed 10% NOS and control diets (Fig. 3).

Table 4: Total protein and amino acid composition of njangsa seed

Composition (%)	
Crude protein[a]	27.00
Amino acids[b]	
Alanine	1.25
Arginine	3.54
Aspartic acid	2.59
Cystine	0.97
Glutamic acid	4.21
Glycine	1.40
Histidine	0.61
Isoleucine	1.13
Leucine	1.64
Lysine	0.71
Methionine	0.58
Phenylalanine	1.26
Proline	1.20
Tryptophan	0.13
Serine	1.49
Threonine	1.05
Tyrosine	0.75
Valine	1.98

[ab]Analyzed by Midwest Laboratories, Inc., Omaha, Nebraska

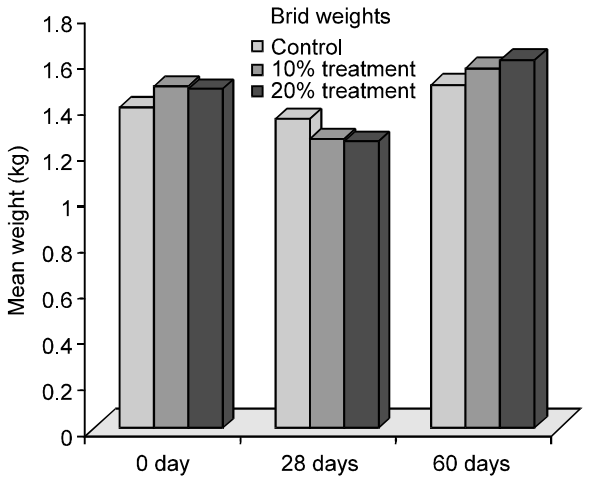

Fig. 2: The mean weight of Birds at three time intervals in each treatment group.
[a,b,c]Means in the same row with different superscripts differ ($p<0.05$). Control = layer diet; 10% NOS; 20% NOS

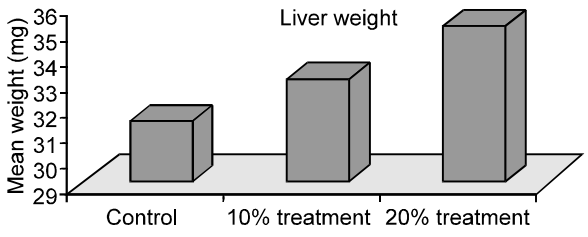

Fig. 3: The mean weight in grams of bird's liver taken at time of extraction in each treatment group ($p>0.05$)

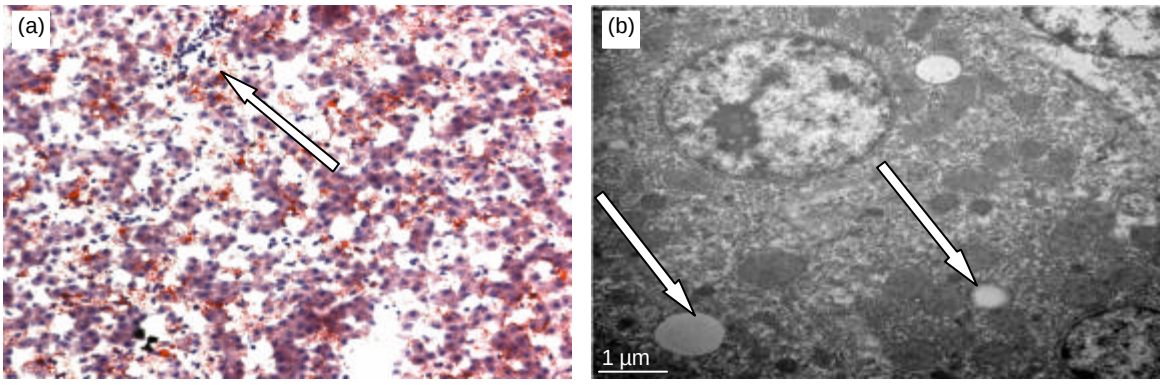

Fig. 4: (a) Lipid droplets in control sample stained with Oil red O and Harris hematoxylin. (b) Lipid droplets in 0% NOS sample observed with TEM

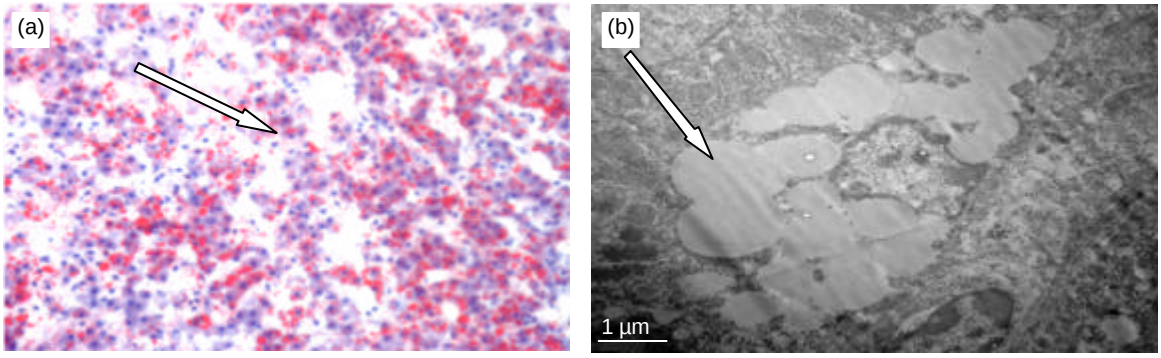

Fig. 5: (a) Lipid droplets in 10% NOS sample stained with Oil red O and Harris hematoxylin. (b) Lipid droplets in 10% NOS sample observed with TEM

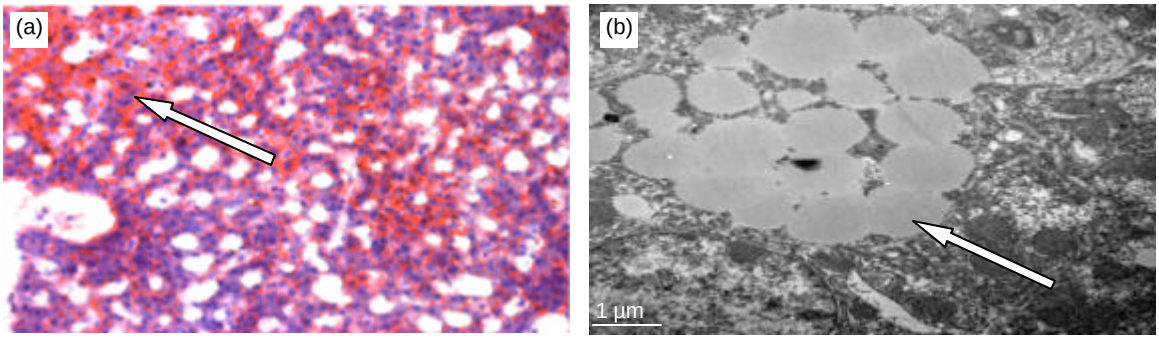

Fig. 6: (a) Lipid droplets in 10% NOS sample stained with Oil red O and Harris hematoxylin. (b) Lipid droplets in 20% NOS sample observed with TEM

Histological studies of liver tissues with oil red stain (Fig. 4a, 5a, 6a) and Electron Transmission Microscope (ETM) (Fig. 4b, 5b, 6b) showed higher ($p<0.05$) accumulation of lipid droplet in the liver from birds fed NOS diets (Fig. 5a, 5b and Fig. 6a, 6b) than in liver from birds fed control diet (Fig. 4a and 4b). In addition, the intensity of the stained lipid droplets was measured by scoring the color intensity on all liver tissues stained with oil red (Table 5). We observed that about 71% of the liver tissues from birds fed control diet had a weak (0) stain, while 21% had a mild (±) stain and 8% had a strong (+) stain. It was also observed that about 57% of liver tissues from birds fed 10% NOS diet had a strong (+) stain, 29% had a mild (±) stain and 14% weak (0) stain. Approximately, 86% of liver tissues from birds fed 20% NOS diet had strong (+) stain, 7% had mild (±) stain and 7% had weak (0) stain (Table 5).

Table 5: Intensity of the lipid stained with oil red O in sections of the liver samples

	Scores (%)		
Treatment group	+	±	0
Diet 1 (Control, 0% NOS[a])	7.14	21.40	71.43
Diet 2 (10% NOS supplement)	57.14	28.57	14.29
Diet 3 (20% NOS supplement)	85.71	7.14	7.14

[a]Njangsa oil seed. Semi-quantitative visual technique (+ = Strong; ± = Mild; 0 = Weak)

mRNA expression by Real-time RT-PCR: Levels of mRNA of the *SCD-1* gene were determined using real-time RT-PCR. The amplicon is shown in Fig. 7. All samples were amplified with high fluorescence intensity as shown on optics graph (Fig. 8). Dilution end point standard curves were obtained for all samples using a representative sample with the lowest Ct value (Fig. 9). The high correlation coefficient (R^2) values obtained indicates the precision of the quantization technique and correlates the Ct values obtained to the number of RT-PCR units, hence the expression level of the SCD-1 gene. Based on the RT-PCR units obtained, comparison between the three groups showed no significant differences among the treatment groups. However, we

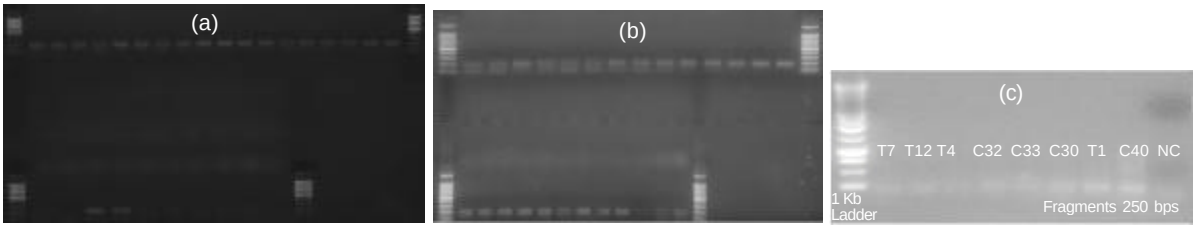

Fig. 7: Gel electrophoresis of RT-PCR products of 0%NOS (a); 10% (1st line) and 20% (2nd line) NOS (b) and beta-action (c)

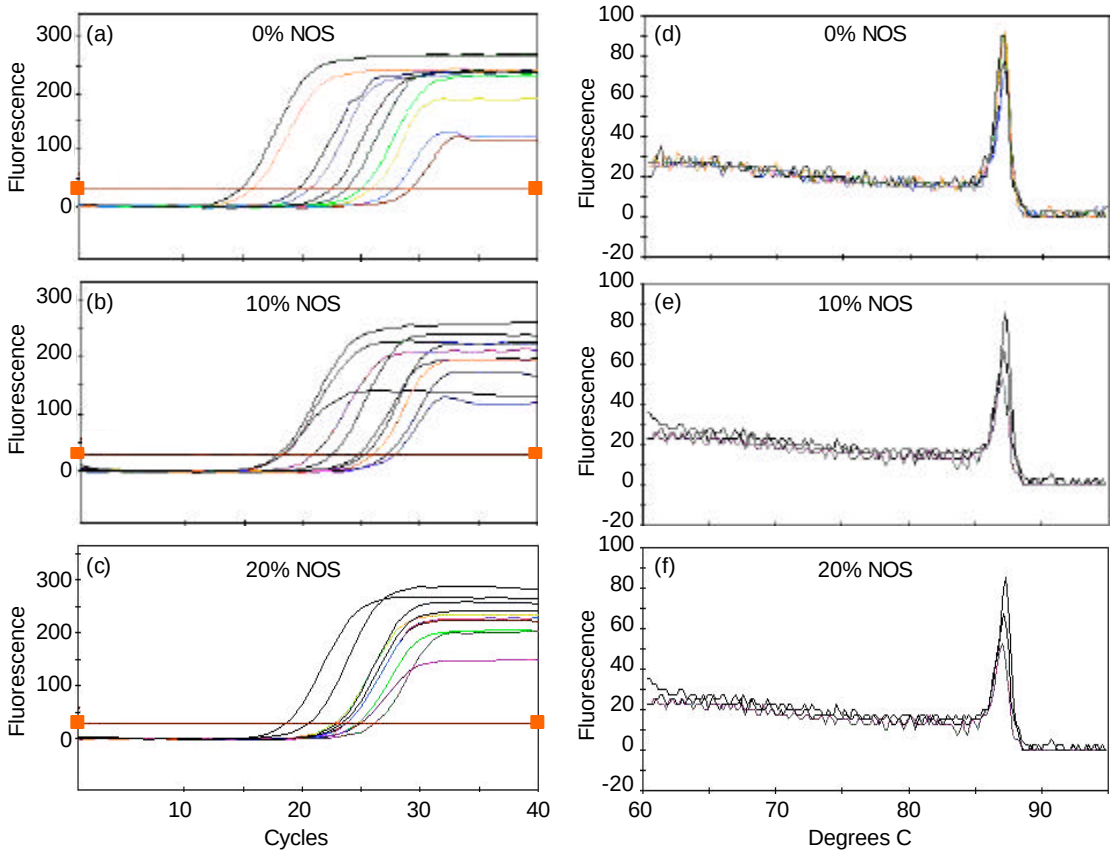

Fig. 8: Results of real-time RT-PCR amplification of SCD-1 from hen liver fed Njangsa Oil Seed (NOS). Optics graphs (a, b and c) show amplicon production. First-derivative melt graphs (d, e and f) show corresponding melting temperatures for each treatment group

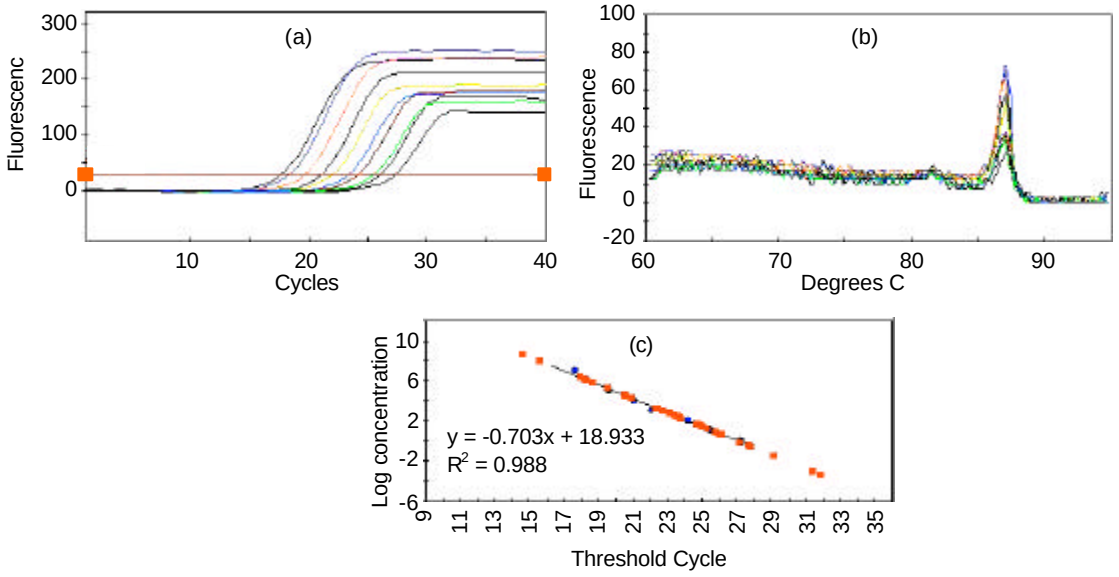

Fig. 9: Optics graph (a) of sample with the lowest Ct value used to obtain standard curve. First-derivative melt graphs (b) of the lowest Ct value show corresponding melting temperatures and the standard curve (c)

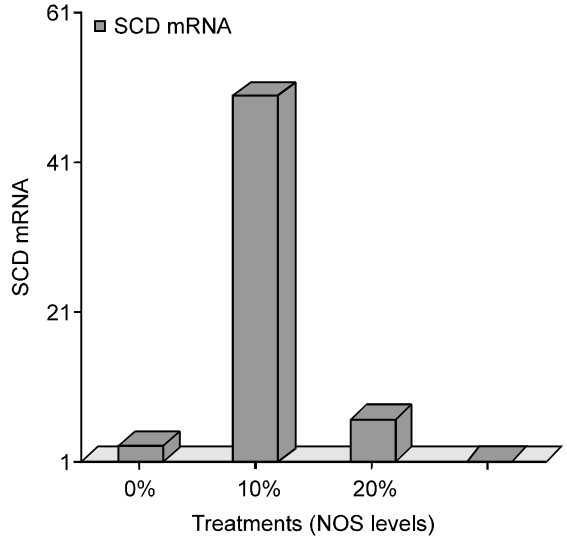

Fig. 10: Effects of Njangsa Oil Seed (NOS) on SCD-1 mRNA ($p>0.05$)

observed that SCD-1 mRNA levels were higher ($p>0.05$) in the liver of birds fed the 10% NOS diet compared with the control and 20% NOS fed birds (Fig. 10).

DISCUSSION

The American Heart Association (AHA) and National Academy of Sciences/Institute of Medicine have recommended that consumers should substitute polyunsaturated fatty acids, particularly omega-3 fatty acids (mainly from marine fatty acids) for saturated fatty acids in order to prevent risk factors associated with heart disease/cardiovascular disease (Krauss *et al*., 2000). In order for consumers to meet the recommended levels of omega-3 fatty acids, it is estimated that a fourfold increase in fish consumption in the United States is necessary (Kris-Etherton *et al*., 2000; Gebauer *et al*., 2006). This study has demonstrated that Njangsa Oil Seed (NOS) has high levels of omega-3 fatty acids compared to commercial oil seeds, thus making it a promising land-based source of omega-3 fatty to meet the demand for those who do not consume fish. Supplies of marine fish oils are limited, therefore alternative land-based sources such as njangsa oil seeds must be evaluated to meet the growing demand for omega-3 fatty acids.

Pathogenesis of liver steatosis in laying hens remains poorly understood. However, it has been suggested that liver steatosis could result from increased fatty acid synthesis in liver, impaired lipid transport from liver and decreased oxidation of fatty acids. Previous studies reported that chronic ω-3 fatty acid supplementation significantly lowers postprandial Triglyceride (TG) concentrations regardless of the type of fat in test meal (Harris and Connor, 1980; Harris and Windsor, 1991; Khan *et al*., 2002). Harris *et al*. (1997) also reported that fish oil increased preheparin LPL activities and reduced plasma TG concentrations by 36% in hypertriglyceridemic patients. Results from this study showed that NOS supplementation in diet of hen increased fat accumulation in the liver, thus indicating that NOS supplementation may not prevent liver steatosis in laying hen. We also observed that supplementation of njangsa oil seed at 10% level in the diet of laying hen increased expression of SCD-1 gene,

thus indicating that NOS supplementation may up regulate the expression of SCD-1. There appeared to be a positive correlation between hepatic lipid droplets and expression of SCD-1 gene, thus, indicating that supplementation of NOS in diet of laying hen may not prevent development of fatty liver. At 20% NOS supplementation, SCD-1 mRNA levels decreased but the concentration of fat in the liver of birds fed the 20% diet was higher. Our result did not corroborate with those of Jordal *et al.* (2005) who reported that *SCD-1* gene was down regulated in Atlantic salmon (*Salmo salar* L.) fed dietary rapeseed oil for 22 weeks. In a similar study, Besong *et al.* (2010) observed an increase in the expression of apolipoprotein B (Apo B) and Microsomal Triglyceride Transfer (MTP) protein mass in laying hen fed 20% NOS supplemented diet. At 10% NOS level, there appeared to be a strong correlation between hepatic lipid droplets and transcription of SCD-1 gene, suggesting that increased expression of SCD-1 mRNA may enhance hepatic lipid synthesis. Our result was not also consisted with studies with mouse a model that reported that SCD1-deficient ($SCD1^{-/-}$) mice had reduced synthesis of lipids, especially triglycerides (Miyazaki and Ntambi, 2003). Similar studies reported that $SCD1^{-/-}$ mice had low levels of triglycerides in very-low-density lipoprotein and increased plasma high-density lipoprotein cholesterol levels (Cohen *et al.*, 2002; Huuskonen *et al.*, 2001). Even though supplementation of Njangsa Oil Seeds (NOS) in layer diet did not prevent fat accumulation in hen liver in this study, the health benefits of omega-3 fatty acids, specifically eicosapentaenoic acid (EPA, C20:5) in NOS needs to be investigated.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Gary Richards and Mr. Michael Watson of USDA - ARS lab at Delaware State University; Dr. Kirk Czymmek and the Delaware Biotechnology Institute (DBI) for their technical assistance. The authors would also like to thank USDA - NRI and Delaware IDeA Network of Biomedical Research Excellence (INBRE) for providing financial support for this trial.

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