

Niacin supplementation on egg and tissue cholesterol of laying hens

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ABSTRACT

This study was carried out to investigate the effect of niacin supplementation in layers diets on the eggs and tissues' cholesterol of laying hens as niacin affects cholesterol in egg and tissue. A total of 75 laying birds at 24 weeks of age were used in the trial. Four dietary treatment with niacin at 0ppm, 30ppm, 40ppm, and 50ppm were fed to the bird for a period of 20 weeks. Each treatment had 18 birds comprising of 3 replicates of six birds each. Egg and tissue cholesterol were measured by enzymatic colorimetric methods using Randox[®] diagnostic cholesterol. At 30ppm of niacin in the diet of layers, resulted in reduction ($p < 0.05$) in the concentrations of the cholesterol in the heart, thigh, and breast tissues. No significant ($p > 0.05$) differences were noticed in egg cholesterol content when the birds were fed diet containing 50ppm of niacin. Meanwhile, the high-density lipoprotein HDL in egg experienced an increase while there was a reduction in low-density lipoprotein LDL at 50mg level of niacin supplementation. It could be concluded that supplementation of laying hen diets with niacin had no effect on egg, liver and heart cholesterol.

Keywords: Niacin, cholesterol, laying hens

INTRODUCTION

Niacin is a dietary precursor for the synthesis of nicotinamide adenine dinucleotide (NAD) and its phosphorylated derivative NADP, which are the essential coenzymes for the metabolism of nutrients in the body (Bogan and Brenner 2008). NAD (NADH) is extensively used in the catabolism of carbohydrates, fats, proteins, and alcohol to produce energy (Gille *et al.*, 2008). Thus, niacin could be essential for the improvement of growth performance and fat reduction in animals (Yang *et al.*, 2016). The physiological effects of niacin on nutrient utilization and lipid metabolism have been variable to a great degree among animal studies (Grewal *et al.*, 2014; Matte *et al.*, 2016).

The need for further research that will address the attendant carcass fatness because of the current consumer awareness of the related ailment has been advocated (Aletor *et al.*, 1998). Moreover, with the growing concern between the relationship between diet and health, there is an increased emphasis on modification of fat and cholesterol content of edible animal products.

Egg is a popular product from the poultry industry and is an excellent natural source of animal protein and has high comparative advantage over other protein source. It is of such high quality that it is commonly used as a standard for measuring the nutritional quality of other foods. The cholesterol content of eggs is a potential disadvantage for the use of eggs in human

diets, bearing in mind the consumer's fear for cholesterol and heart disease. A reduction of cholesterol content of market eggs therefore becomes understandably a subject of interest to both egg producers and health conscious consumers. However, efforts have been made by many researchers to lower egg yolk cholesterol content. Research areas in which these efforts have been made include use of drugs, manipulation of dietary protein and energy levels of dietary source, supplementation with vitamin A, ascorbic acid, or vanadium and the use of dietary fibre which is known to be hypocholesterolemic. Fibre inclusion however, has shown to reduce egg cholesterol production in hens (Lirette *et al.*, 1993). In the recent time, niacin has been identified as an alternative to the reduction of high level of cholesterol and low density lipoprotein Sullivan (2006). Niacin, also known as vitamin B3 or nicotinic acid, is required for the synthesis of nicotinamide-adenine dinucleotide and nicotinamide-adenine dinucleotide phosphate, two enzymes essential for the metabolism of proteins, carbohydrates and lipids (NSM, 2007). Oloyo, (2002) reported that dietary niacin level of 37.5mg/kg feed was adequate for achieving good nutrient utilization, optimizing productive performance and for maintaining good health. Large doses of these vitamins according to Sullivan (2006) is known to increase the level of high density lipoprotein (HDL) (good) cholesterol in blood, and is often prescribed for patients with low HDL and consequently high risk of heart attack. Studies in animal laboratories have demonstrated behavioural changes when large doses of niacin are given (Sullivan, 2006). Researchers have also reported remarkable improvement in the utilization of nitrogen metabolizable energy, calcium and phosphate when higher dietary niacin levels were used in breeder chicken diets (Leeson *et al.*, 1979). The objective of this study is to determine the effect of niacin

supplementation on eggs and tissue cholesterol levels in laying hens.

MATERIALS AND METHODS

This study was carried out at the Poultry Unit of Teaching and Research Farm, Federal University of Agriculture, Abeokuta, (7°10'N and 32°E) in Odeda Local Government Area Ogun State. A total of 72 laying birds (Harco brown) at 24 weeks old were obtained from a reputable farm. The house and cage used were thoroughly washed and disinfected before arrival of the laying birds. Feed and clean water were supplied *ad libitum* while the other routine vaccination with necessary medication was administered to the birds accordingly. Four Experimental diets were formulated such that the niacin was added to the basal diet at 0, 30, 40, 50mg/kg of feed respectively.

The 72 laying birds were randomly allocated to the four dietary treatments of 18 birds each in a completely randomized design. Each treatment group were further divided into three replicates of 6 birds each. The experiment lasted for 20 weeks.

Sampling for egg cholesterol determination

Twelve eggs (3 per replicate) per treatment sampled at the expiration of the laying period were weighed and cooked by immersion in boiling water for 10 minutes. Yolks were removed, individually weighed and oven-dried at 70°C. Egg total lipid was extracted with chloroform:methanol (2:1 v/v) using the procedure described by Folch *et al.* (1975). Cholesterol determination was done using a commercial test kit for cholesterol analysis (Sigma Chemical Co., St Louis, MO USA). Cholesterol concentration was determined from absorbance read at 500nm using a spectrophotometer.

Tissues and liver samples preparation.

At the end of the experiment, one bird per replicate was selected, making a total of 12 birds, weighed, slaughtered, scalded, plucked and eviscerated. The cut parts such as thigh drumstick, breast muscles, liver, heart and kidney were excised, blotted dry and weighed. The weight was expressed as a percentage of the live weight. Cholesterol content of tissues was determined by enzymatic-calorimetric methods using Radox[®] a diagnostic cholesterol kit

(BIOLAB with code 80106.2 x 100ml cholesterol (HOD –PAP[®]). The cholesterol kits vials (R1 & R2) mixture were added to the extract and then incubated for ten mins in which the colour change from milky to pink. The absorbance of the samples plus the reagents and the standard plus the reagents were read at 480nm spectrophotometer (Jenway[®] against the reagents blank within one hour). Cholesterol content was calculated as:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Standard conc. (200mg/dl)}$$

$$\text{Cholesterol conc. (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Standard conc. (200mg/dl)}$$

Triacylglyceride determination procedure

A commercial test kit used for the triglyceride (triglycerol) determination was Radox[®] diagnostic triglyceride reagent procedure (GPO-PAP Method Radox Laboratory Ltd, UK). This procedure was also based on enzymatic-colorimetric methods (according to manufacturer's manual). The triglyceride was determined after the enzymatic hydrolysis with lipases. The indicator was quinoneimine formed from hydrogen peroxide. The reagent contains 4-aminophenazone, ATP, lipases, glucokinase, glycerol-phosphate oxidase peroxidase and peroxidase.

The kit consist of R₁ (Buffer solution containing 4-chlorophenol and magnesium ions) R₂ is the enzyme reagent, while vial R₃ is the standard solution (200mg/dl). The R₂

vial was emptied into R₁ and reconstituted mixture was left to stand for 10minutes.

1000μL of the reagent (reconstituted reagent) was pipetted into test tubes containing 10μL of the standard and sample extract respectively. The content of the tubes was then mixed gently and incubated for 10 minutes at 37°C for 5 minutes. The milky colouration of the incubated materials changed to pink. The content in each test tube was immediately poured into two different glass cuvettes. Absorbance of the incubated sample extract plus reagent (A_{sample}) and the stand plus reagent (A_{standard}) were read against the blank in Jenway[®] spectrophotometer at a wavelength of 500nm.

Calculations:

$$\text{Triglyceride conc. (mg/dL)} = \frac{\text{A sample}}{\text{A standard}} \times 200\text{mg/dL}$$

Further calculations were done to convert mg/dL to mg/g of the blood samples .

Statistical Analysis

Data obtained for tissues and egg cholesterol content were subjected to general linear model in analysis of variance (Steel and Torrie, 1980), and Significant means were

separated using Duncan's Multiple Range Test (Duncan 1955).

The analysis was based on this model

$$X_{ij} = \mu + T_i + E_{ij}$$

Where X_{ij} = observation in the i th treatment

μ = Population mean

T_i = Effect of niacin level

E_{ij} = random error

RESULTS

Effect of niacin supplementation on the cholesterol content of egg.

Table 1 shows the effects of niacin supplementation on the cholesterol content of

egg. The results showed that the HDL was significantly ($P < 0.05$) influenced by the level of niacin supplemented in the diets. The highest value for the HDL was recorded at 40ppm and was significantly different from the control. The cholesterol, triglycerides and LDL were not significantly ($P > 0.05$) influenced by the level of niacin supplementation. However, as the level of niacin increased, the level of cholesterol decreased although no significant ($p > 0.05$) difference was observed.

Table 1: Effect of niacin supplementation on egg cholesterol content of laying hens

Parameters	0ppm	30ppm	40ppm	50ppm	SEM
Cholesterol (mg/g)	15.84	13.48	14.19	13.81	0.79
Triglyceride (mg/g)	7.52	8.65	8.44	7.81	0.29
HDL (mg/g)	1.87 ^b	1.94 ^{ab}	1.98 ^a	1.96 ^{ab}	0.12
LDL (mg/g)	4.18	3.42	3.91	4.32	0.18

^{abc} means on the same row having different superscripts are significantly different ($p < 0.05$).

Effect of niacin supplementation on the tissue cholesterol content of laying hens.

Table 2 shows the effects of niacin supplementation on the tissue cholesterol content of laying hens. The results of the study showed that the liver and the heart cholesterol were not significantly ($P > 0.05$) influenced by niacin level. However, thigh and breast cholesterol were significantly ($P < 0.05$) influenced by niacin level. Thigh and breast cholesterol had highest value at 50ppm and was significantly different from the control group. Liver and breast triglyceride were significantly ($P < 0.05$)

increased at 30ppm with highest value of 14.70mg/g and 18.93mg/g at 50ppm and was statistically different from the control. The heart and thigh triglycerides were not significantly ($p > 0.05$) reduced. Thigh and breast HDL were significantly ($P < 0.05$) increased, with the highest value for the control and 50ppm for thigh and the same trend was observed for breast HDL. The liver and heart LDL were significantly ($P < 0.05$) increased with the highest value recorded at 40ppm and was significantly different from the control. The thigh and breast LDL were not significantly ($P > 0.05$) reduced.

Table 2 Effect of niacin supplementation on the cholesterol content of tissues of laying hens

Parameters	0ppm	30ppm	40ppm	50ppm	SEM
Liver	6.34	8.04	6.67	6.20	0.50
cholesterol(mg/g)					
Liver triglyceride	11.57 ^b	14.70 ^a	12.20 ^{ab}	13.71 ^{ab}	0.54
(mg/g)					
Liver HDL (mg/g)	1.59	1.43	1.34	1.17	0.10
Liver LDL (mg/g)	2.17 ^c	2.62 ^{ab}	2.75 ^a	2.54 ^b	0.07
Heart cholesterol	6.67	4.31	5.23	5.25	0.45
(mg/g)					
Heart triglyceride	17.89	17.38	21.97	16.50	1.01
(mg/g)					
Heart HDL (mg/g)	1.05	1.26	1.15	1.08	0.04
Heart LDL (mg/g)	2.32 ^c	2.21 ^d	3.98 ^a	2.65 ^b	0.09
Thigh cholesterol	4.31 ^b	4.31 ^b	6.03 ^a	6.58 ^a	0.37
(mg/g)					
Thigh triglyceride	12.08	12.96	13.07	11.53	14.0
(mg/g)					
Thigh HDL (mg/g)	1.05 ^a	1.01 ^b	1.01 ^b	1.05 ^a	0.01
Thigh LDL (mg/g)	2.76	2.83	2.98	2.78	0.10
Breast	6.19 ^{ab}	3.81 ^b	6.35 ^{ab}	8.55 ^a	0.63
cholesterol(mg/g)					
Breast	10.80 ^b	16.65 ^a	11.02 ^b	18.93 ^a	1.26
triglyceride(mg/g)					
Breast HDL	1.09 ^a	1.10 ^a	1.08 ^b	1.05 ^c	0.01
(mg/g)					
Breast LDL (mg/g)	2.50	2.34	2.79	2.81	0.09

^{abc} means on the same row having different superscripts are significantly different ($p < 0.05$)

DISCUSSION The cholesterol content of egg was not significantly influenced by the dietary level of niacin supplementation, this is in contrast with the report of LEESON et al 1991 but in agreement with Leeson *et al*, 1991 who reported that niacin level up to 1.022g/kg which is more closely stimulates therapeutic levels used for humans failed to significantly affect egg cholesterol content. It was very difficult to compare egg or yolk cholesterol levels between research reports because not only the analytical methods differ but the anti-cholesterolemic agent also differ (i.e. the test ingredients, pharmacological substances and the diet compounded. Cholesterol and steroid is

known to be a key precursor of steroid hormones and steroid hormones are involved in the control of reproduction in females. It is apparent that cholesterol level might be related to production variables. The positive correlation between the various cholesterol contents and the trait of economics importance indicated that selection for low cholesterol level would result in concomitant reduction in the weight, feed intake and egg size.

The triglyceride and LDL were not by the levels of niacin, there was a reduction in LDL which indicated that there is negative correlation between the niacin levels and the

LDL reduction. However, HDL was affected, it increased in the values as the niacin levels increases. This implied that niacin appears to raise HDL cholesterol by reducing hepatic, apolipoprotein A-I clearance and enhancing reverse cholesterol transport.

The results from the cholesterol content of tissues showed that the liver triglyceride, liver LDL, heart LDL, thigh cholesterol, thigh HDL, breast cholesterol, triglyceride and breast HDL were significantly affected by the level of niacin supplementation, while, the liver cholesterol, liver HDL, heart cholesterol, thigh triglyceride, LDL and breast LDL were not significantly affected by the levels of niacin, this result agreed with Leeson *et al*, 1991 who reported no significant effect when birds were fed 22mg/kg of niacin on the liver cholesterol content. The significant depression in the heart, thigh and blood cholesterol at 30ppm niacin supplementation in laying hens is in agreement with earlier report of (Waldroup *et al*, 1984) and it is a desirable feature. However, the amount of the tissue lipids/cholesterol has been related to the levels of dietary lipid supplied in the blood (Paik *et al*, 1993, since the leg and the breast muscle are neither the synthesis site nor the storage site of the lipid. It could be concluded that supplementation of laying hens diets with niacin had no effect on egg, liver and heart cholesterol.

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