SERUM AND EGG YOLK CHOLESTEROL LEVELS OF BIRDS FED DIETARY GARLIC AND NATURAL COCOA POWDER

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Abstract

This study was conducted to evaluate the effect of Natural cocoa powder (NCP) and Garlic on the laying capacity and some egg quality characteristics of a commercial laying flock. A total of 270 Golden sex linked layers in their 8th week of lay were used for the study. The birds were allowed to acclimatize for a week and then randomly divided into nine treatment groups with thirty birds per group. Each treatment group was replicated twice. The birds in T1, T2, T3, T4, T5, T6, T7, T8, and T9 were fed basal diet (layer's mash) supplemented with natural cocoa powder (NCP) and garlic powder (GP) at 0gNCP and 0gGP, 0gNCP and 50gGP, 0gNCP and 100gGP, 50gNCP and 0gGP, 50gNCP and 50gGP, 50gNCP and 100gGP, 100gNCP and 0gGP, 100gNCP and 50gGP, and 100gNCP and 100gGP per 100kg diet respectively. T1 served as the control group. The experimental diet and water were provided ad-libitum. Eggs were collected and the effect of dietary treatments on egg quality characteristics and cholesterol levels determined. Results showed that supplementation of Natural cocoa powder and garlic had significant ($P<0.05$) effects on the hen day production, serum and yolk cholesterol, and egg, yolk, and albumen weight. The highest egg, yolk and albumen weight were obtained for birds in T2 (68.95, 15.40, and 45.35g) and T3 (65.40, 16.55, and 39.90g) which were supplemented respectively with only 50 and 100g of GP and were significantly higher than the controls (64.2, 15.95, and 40.45g). Eggs from birds in T4 and T7 supplemented with only 50 and 100g of NCP were similar ($p>0.05$) to the control. Combined supplementation of NCP and GP significantly increased yolk and albumen weight at lower doses which was significantly lowered at higher doses. Serum and yolk cholesterol were significantly decreased in all the treatment groups when compared with the control. The results show that both natural cocoa and garlic powder have great cholesterol reducing potential but increased egg, yolk, and albumen weight could be achieved by GP alone or with NCP at a combined dietary level of 50:50g.

Introduction

In the recent years, cholesterol has been stigmatized by Nigerians especially in the nutrition and food processing sectors of the country where most packaged food product has been branded 'Cholesterol free' consequently promoting its acceptance and marketability to the general public. This is due to the concern that plasma cholesterol and low density lipoprotein (LDL) may be closely associated with atherosclerosis and consequently coronary heart disease and death (Waldroup et al., 1986; Kamruzzaman et al., 2012). A correlation has also been established to exist between dietary cholesterol and plasma cholesterol levels in humans (Schonfeld et al., 1982) though a study by Weggemans et al. (2001) showed that dietary cholesterol increases LDL-cholesterol but is without effect or increases HDL-cholesterol. On the recommendation of not more than 300mg per day and three to four whole egg yolk consumption per week for a person by the American Heart Association, consumption of high cholesterol food and eggs to some extent have been significantly lowered (Kamruzzaman et al., 2012). The negative concerned association with yolk cholesterol and consumers interest in their health have provoked researches to reduced cholesterol levels in egg yolk (Reddy et al., 1991) using animal fat free and hormone-free diets (Cherian et al., 2002). Consequently, many studies have been carried out using natural substrates as cholesterol lowering agents (Santoso et al., 2005). Garlic (Allium *sativum*) which is well distributed and widely employed in different parts of the world as a cooking spice as well as for prophylactic and therapeutic courses of many diseases spanning from infections to cardiac disease (Konjufca et al., 1997). Garlic supplementation in diets of animals have been suggested to limit cholesterol and fatty acids synthesis in the liver (Qureshi et al., 1983; Yel and Liu, 2001) through the inhibition of rate limiting enzymes in the reactions and Allicin (the chemical substance responsible for the characteristic garlic smell) is believed to be the active compound in this (Azeke and Ekpo, 2008). Warshafsky et al., (1993) suggested daily consumption of 1.5 to 3g of fresh garlic for 2 to 6 months can lower cholesterol by 9%($0.59mmol^{-1}$). Plasma cholesterol and hepatic total lipid and

cholesterol were reduced when dietary garlic was fed to laying hens for 3weeks (Sharma et al., 1979), and when dietary garlic paste was fed though in a dose dependent manner (Chowdhury et al., 2002). However, Birrenkott et al., (2000) demonstrated that 3% garlic powder diet did not significantly lower levels of cholesterol in egg yolk and serum.

Cocoa powder from the seed of Theobroma cacao is known to be rich in polyphenols, such as the procyanidin monomers (Catechin and epicatechin) and oligomers, a B-type procyanidins that are linked by $C_4 - C_8$ (Hammerstone et al., 1999; Hatano et al., 2002). Monomers of procyanidins have been evidently suggested to prevent coronary heart diseases (Arts et al., 2001). Also dietary supplementation with cocoa or its procyanidins fractions have been shown by several studies to lower significantly the susceptibility of LDL to oxidation in human (Wang et al., 2000; Osakebe et al., 2001; Wan et al., 2001) and high cholesterolemic rabbits (Osakebe et al., 2000). Osakebe and Yamagishi, (2009) reported lowered plasma total cholesterol levels in rats fed on a high cholesterol diet and cocoa powder. However, limited studies have been done to determine the effect of combined treatment of garlic and cocoa on the egg characteristic, serum and yolk cholesterol level in poultry hence, this present study was conducted to evaluate the effect of cocoa powder supplementation in hens' diet either alone or in combination with garlic on their production performance, egg quality characteristics and serum and yolk cholesterol levels.

Materials And Methods

Location and duration of study: The study was carried out in the poultry unit of the Animal Science teaching and research farm, University of Nigeria, Nsukka. The study lasted for a period of eight (8) weeks.

Procurement of test ingredients: Garlic bulb were purchased from the local commodity market (ogige) in Nsukka Enugu state, Nigeria. They were first cut into smaller sizes and sundried for 4 days. After drying, they were then grinded into powdery form using automated grinding machine before packaging for use. The natural cocoa powder was procured from the National Cocoa Research Institute (NCRI) in Ibadan, Oyo state, Nigeria.

Management of experimental animals

A total of 270 Gold linked layers in their 8th week of lay were randomly assigned to 9 treatment

Table 1: Formula and chemical composition of the experimental diets (%)

Ingredients	T1	T2	T3	T4	T5	T6	T7	T8	T9
Maize	42.59	42.59	42.59	42.59	42.59	42.59	42.59	42.59	42.59
Wheat offal	19.81	19.81	19.81	19.81	19.81	19.81	19.81	19.81	19.81
Palm kernel cake	13.87	13.87	13.87	13.87	13.87	13.87	13.87	13.87	13.87
Soybean meal	19.81	19.81	19.81	19.81	19.81	19.81	19.81	19.81	19.81
Bone meal	1.98	1.98	1.98	1.98	1.98	1.98	1.98	1.98	1.98
Oyster shell	1	1	1	1	1	1	1	1	1
Lysine	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vit. Mineral premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Enzyme toxin binder	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Biomin	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Total	100	100	100	100	100	100	100	100	100
Supplementations									
NCP (g)	0	0	0	50	50	50	100	100	100
Garlic powder (g)	0	50	100	0	50	100	0	50	100
Calculated analysis									
M.E (Kcal/Kg)	2700	2700	2700	2700	2700	2700	2700	2700	2700
Crude protein (%)	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5
Crude fibre (%)	5.49	5.49	5.49	5.49	5.49	5.49	5.49	5.49	5.49

*Each kg of layer premix contains: vit.A, 10,000IU; vit. D3, 2,500IU; vit. E, 20mg; vit. K3, 3mg; thiamine, 2mg; riboflavin, 5mg; pyridoxine, 5mg; vit. B12, 0.015mg; Nicotinicacid, 40mg; Pantothenicacid, 12mg; folicacid, 0.75mg; biotin, 0.05mg; Vit. C, 100mg; manganese, 70ppm; zinc, 60ppm; iron, 60ppm; iodine, 1ppm; copper, 8ppm; selenium, 0.25ppm; cobalt, 0.15ppm

groups replicated twice with 15 hens per replicate in a completely randomized design. The birds in T1, T2, T3, T4, T5, T6, T7, T8, and T9 were fed basal diet (layer's mash) supplemented with natural cocoa powder (NCP) and garlic powder (GP) at 0gNCP and 0gGP, 0gNCP and 50gGP, 0gNCP and 100gGP, 50gNCP and 0gGP, 50gNCP and 50gGP, 50gNCP and 100gGP, 100gNCP and 0gGP, 100gNCP and 50gGP, and 100gNCP and 100gGP per 100kg diet respectively. Birds in T1 group served as the control. The laying hens used for the study were up to date in their vaccination programme and had similar sizes and performances. They were acclimatized for a week before commencing the 8-week trial. The pens were thoroughly washed and disinfected before the introduction of the experimental birds. The egg weight, length and diameter and the daily egg production were recorded for the period of the experiment. At the end of the 8 weeks trial, an egg was collected from each of the birds and the egg quality characteristics of egg, yolk, albumen, and shell weight, and egg length and diameter were measured and recorded. The serum and yolk cholesterol were determined using RANDOX® Cholesterol assay kit. The Experimental diet and water were supplied ad libitum to the birds and proper management practices were followed. The diet used for the study was formulated and the percentage compositions are presented in table 1.

Data collection and analysis: Data obtained from the study was analyzed using analysis of variance in SPSS (version 20). Significantly different means were seperated using Duncan's new multiple range test.

Results And Discussions

The result of the effect of natural cocoa powder and garlic powder on egg characteristics and hen day production of birds are shown in table 2.

Table 2: Effect of natural cocoa powder and garlic powder supplementation on Egg characteristics an d hen day production of birds

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	T9
Egg weight (g)	0.37^{c}	68.95 ± 0.47^{a}	65.40 ± 0.42^{bc}	62.40 ± 0.63^{dc}	66.52 ± 2.69^{b}	61.25 ± 2.48^{e}	62.80 ± 0.42^{d}	64.49 ± 0.42^{c}	64.49 ± 0.11^{c}
Egg length (cm)	5.82 ± 0.42^{c}	5.96 ± 0.63^{a}	5.89 ± 0.14^{b}	5.90 ± 0.13^{ab}	5.89 ± 0.58^{b}	5.85 ± 0.18^{bc}	5.83 ± 0.21^{c}	5.83 ± 0.04^{c}	5.97 ± 0.03^{a}
Egg diameter (cm)	4.55 ± 0.01^{c}	4.76 ± 0.13^{a}	4.65 ± 0.09^{b}	4.57 ± 0.08^{c}	4.59 ± 0.02^{bc}	4.47 ± 0.07^{d}	4.49 ± 0.06^{ed}	4.52 ± 0.03^{c}	4.51 ± 0.07^{cd}
Weight of shell (g)	7.70 ± 0.00^{b}	8.10 ± 0.21^{ab}	8.55 ± 0.37^{a}	7.10 ± 0.11^{c}	7.75 ± 0.69^{b}	7.25 ± 0.26^{c}	7.50 ± 0.42^{bc}	7.15 ± 0.47^{c}	8.30 ± 0.63^{a}
weight of yolk (g)	15.95 ± 0.26^{b}	15.40 ± 0.42^{c}	16.55 ± 0.47^{a}	15.75 ± 0.16^{bc}	16.50 ± 0.00^{a}	15.55 ± 0.90^{c}	15.45 ± 0.16^{c}	16.05 ± 0.16^{b}	16.50 ± 0.42^{a}
Weight of albumen (g)	40.45 ± 0.26^{c}	45.35 ± 0.9^{a}	39.90 ± 0.11^{c}	39.85 ± 0.05^{c}	41.70 ± 2.11^{b}	38.55 ± 1.85^{d}	40.25 ± 0.47^{c}	41.55 ± 0.79^{b}	40.50 ± 0.95^{c}
Hen day production (%)	59.12 ± 0.92^{c}	55.00 ± 0.00^{d}	66.00 ± 4.22^{a}	58.50 ± 1.50^{c}	55.13 ± 3.29^{d}	56.00 ± 1.06^{d}	53.50 ± 0.53^{d}	62.00 ± 0.00^{b}	52.00 ± 0.00^{d}
Serum cholesterol (mg/dl)	156.53 ± 27.50^{a}	106.53 ± 25.21^{b}	106.52 ± 29.79^{b}	113.3 ± 1.58^{b}	104.52 ± 13.57^{b}	91.31 ± 13.75^{bc}	102.18 ± 2.29^{b}	76.09 ± 6.03^{c}	79.57 ± 7.68^{c}
Yolk cholesterol (mg/dl)	2454.55 ± 95.83^{a}	2000.00 ± 0.00^{bc}	1954.55 ± 36.39^{bc}	2018.19 ± 172.49^{b}	1600.00 ± 134.15^{e}	1909.09 ± 95.83^{c}	1772.73 ± 413.74^{d}	1700.00 ± 86.83^{d}	1718.00 ± 9.58^{d}

^{abcdef}Means on the same row with different superscripts are significantly (P<0.05) different. T1 represents the contro l group; T2, 0g NCP and 50g GP; T3, 0g NCP and 100g GP; T4, 50g NCP and 0g GP; T5, 50g NCP and 50g GP; T6, 50g NCP and 100g GP; T7, 100g NCP and 0g GP; T8, 100g NCP and 50g GP; and T9, 100g NCP and 100g GP supplementation each in 100kg diet

Egg characteristics and hen day production

The result showed that egg weight, egg length, egg diameter, yolk weight, albumen weight, shell weight, hen day production and serum and yolk cholesterol were significant (p<0.05) different among the treatment groups. The highest values for egg, yolk and albumen weight were obtained for birds in T2 (68.95, 15.40, and 45.35g) and T3 (65.40, 16.55, and 39.90g) which were supplemented respectively with only 50 and 100g of GP. These values were significantly higher than those of the control group (64.2, 15.95, and 40.45g). Eggs from birds in T4 and T7 supplemented with only 50 and 100g of NCP were similar (p>0.05) to the control. Combined supplementation of 50g NCP and 50GP also

significantly ($p<0.05$) increased egg, yolk and albumen weight (66.52, 16.5, and 41.70) when compared to the control group but was also significantly ($p<0.05$) than those of T2 and T3. Also, the hen day production (%) was highest for the hens in T3 (0NCP/100GP) which was significantly ($p<0.05$) higher than those of the other groups. Significantly ($p<0.05$) higher hen day production than observed for the control was shown for T8 (100NCP/50GP) group. Other levels of combinations of NCP and GP were seen to be either similar or significantly lower than those of the control in all the measured egg characteristics. The results of this study were similar to the reports of Yalcin et al. (2006) that dietary supplementation of 5 or 10% garlic powder showed numerical increase in hen day performance. In addition, Olubamiwa et al. (2000) reported no adverse effect of cocoa powder at low inclusion levels, although, Odunsi et al. (1995) and Emiola et al. (2011) reported reduced feed intake and weight gain when cocoa product was fed at high levels of 100g per kg diet. Furthermore, similar result as obtained in this study for egg production was reported for chicken (Khan et al., 2008) and Japanese quails (Samanta and Day, 1991) when garlic supplemented diet was fed. However, contrary to these findings Reddy et al. (1991), Chowdhury et al. (2002) and Khan et al. (2008) reported that egg weight and mass were not affected by dietary garlic supplementation. The improvement in egg quality characteristics and egg production reported for hens in garlic powder only supplemented groups (T2 and T3) was attributed to its allicin content. Allicin, which is the active ingredient present in garlic have been reported to inhibit the growth of pathogenic bacteria and aflatoxin producing fungi as well as enhancing food consumption, utilization and consequently productivity (Chowdhury et al., 2002). The lowered egg quality characteristics observed for the cocoa treated group was due to the toxicity of its theobromine content which appears to increase with increasing dietary intake with the resultant decreased feed intake and nutrient availability (Emiola et al., 2011).

Serum and yolk cholesterol levels were also observed to significantly ($p<0.05$) differ among the treatment groups. Serum cholesterol levels for the hens in T1, T2, T3, T4, T5, T6, T7, T8 and T9 were 156.53 ± 27.50, 106.53 ± 25.21, 106.52 ± 29.79, 113.3 ± 1.58, 104.52 ± 13.57, 91.31 ± 13.75, 102.18 ± 2.29, 76.09 ± 6.03, and 79.57 ± 7.68 respectively. The respective yolk cholesterol levels were 2454.55 ± 95.83, 2000.00 ± 0.00, 1954.55 ± 36.39, 2018.19 ± 172.49, 1600.00 ± 134.15, 1909.09 ± 95.83, 1772.73 ± 413.74, 1700.00 ± 86.83, and 1718.00 ± 9.58 for hens in T1, T2, T3, T4, T5, T6, T7, T8 and T9. From the study, it was observed that as the levels of NCP and GP increased, the cholesterol levels were found to decrease when NCP and GP were supplemented alone or in combination. The least values were obtained for cocoa treated groups. The results of the present study was contrary to that of Birrennott et al., (2002) but similar to those of other authors who reported that serum and yolk cholesterol levels were lowered by garlic supplementation (Myung et al., 1982; Qureshi et al., 1983; Chowdhury et al., 2002; Lim et al., 2006; Azeke and Ekpo., 2008). Allicin component of garlic has been reported to inhibit to inhibit hepatic HMG-CoA reductase, cholesterol 7α hydrogenase, and fatty acid synthesis in chicken (Quereshi et al., 1983). Aslo, Taghyani et al. (2011) reported lowered serum cholesterol in broiler chicks after a 42 days feeding trial of cocoa powder. However, Ruzadi et al., (2005) and Osakebe and Yamashi, (2009) reported a reduction in total plasma cholesterol levels in cholesterolemic rats fed diet supplemented with cocoa powder. Similar reports were made with rabbits for cocoa procyanidins and for tea and its Catechin components (Ikeda et al., 1992) in rats. These authors attributed this effect to the hypocholesterolemic effect to cocoa powder which may inhibit intestinal absorption. Cocoa is a rich source of polyphenols (the flavan-3-ol monomers Catechin and epicatechin and procyanidins), although little information is available about the absorption, metabolism, and antioxidant activity of cocoa flavonoids in human (Wan et al., 2001). These antioxidants in cocoa have the ability to increase the synthesis of nitric oxide (NO) which can cause vasodilation and consequently clearing and limiting the deposition of excess cholesterol in blood vessels (Toghyani et al., 2011). The cholesterol lowering ability of the combined NCP and GP (in T5) reveals a synergistic interaction of the two additives in limiting the quantity of very low density lipoprotein (egg cholesterol) which according to Kamruzzman et al. (2012) are synthesized in the liver and transported to the ovary for yolk formation via the plasma. Future research should hence be channeled to provide more information on the mechanism of interaction of cocoa and garlic in lowering serum and yolk cholesterol.

conclusion

It was concluded that dietary supplementations of garlic powder alone or in combination with natural cocoa powder at levels of 50gNCP and 50GP improved egg quality while garlic powder and natural cocoa powder lowered both serum and

yolk cholesterol levels.

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