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Research Article
Raw *Allium sativum* as Performance Enhancer and Hypocholesterolemic Agent in Laying Hens

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Abstract

Background and Objective: Nutritionally, poultry eggs are referred to compete protein owing to their amino acid profile. However, consumption of egg is usually with caution because they are rich sources of cholesterol in human diets. Consumption of cholesterol-rich diet has a positive correlation with increased serum total and low-density lipoprotein cholesterol (LDL) concentrations both in human and animals. High serum total and LDL levels have been associated with increased deposition of fat around the arterial walls which could result in increased incidence of atherosclerosis and coronary heart diseases. This study was carried out to investigate raw *Allium sativum* as performance enhancer and hypocholesterolemic agent in laying hens. **Materials and Methods:** The efficacy of raw garlic (RG) as performance booster and cholesterol-lowering agent in laying hens was investigated in a 20 weeks feeding trial. Eighteen weeks old Isa Brown hens (n = 180) were randomly distributed to 6 dietary treatments. Each dietary treatment consisted of 6 replicates of 5 birds each. The serum samples were analyzed for total cholesterol (TC), triglycerides (Tg) and high-density lipoprotein cholesterol (HDL) using randox kit. Data were analyzed by one-way analysis of variance (ANOVA) using SPSS version 16 and the means were separated using Duncan's multiple range test of the same software. **Results:** Five percent RG inclusion resulted in reduced feed intake in the experimental laying hens while 2% inclusion of RG had the highest hen-house production. The highest hypocholesterolemic effect of RG was observed in birds fed 3% RG (79 mg dL^{-1}), followed by birds fed with 2, 4, 1 and 5% RG, respectively. The effect of inclusion of RG in the diets of laying on TC was similar for birds fed with 2, 3 and 4% RG. **Conclusion:** The RG inclusion improved high-density lipoprotein cholesterol but reduced total cholesterol contents in the experimental animals.

Key words: *Allium sativum*, atherosclerosis, cholesterol, lipoprotein, raw garlic

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry eggs have been held up as a powerhouse of nutrition. This reputation is due to egg's exceptional nutritional profile as a nutrient-dense food containing high quality protein and substantial amounts of many essential vitamins and minerals. Unfortunately, their position on the nutrition pedestal fell with the discovery that they are a major source of dietary cholesterol[1].The cholesterol is deposited mainly in the egg yolk, which represents about one-third of the egg content by weight.

Despite the important contribution of eggs in human diets and the fact that eggs are low-cost sources of protein and certain vitamins, consumption of egg is usually with caution because poultry eggs are rich sources of cholesterol in human diets. Consumption of cholesterol rich diet has a positive correlation with increased serum total and low-density lipoprotein cholesterol (LDL) concentrations both in human and animals[2-3]. High serum total and LDL levels have been associated with increased deposition of fat around the arterial walls. This results is increased incidence of atherosclerosis and coronary heart diseases[4]. Among 514 Australian aborigines, consumption of >2 eggs/week was associated with a 2.6 times greater risk of coronary artery disease in a prospective analysis[5]. While a report of meta-analysis by Weggemans *et al*.[6] indicated that consumption of an egg/day increases heart disease risk by 2.1%.

Garlic (*Allium sativum*) is widely distributed and used in all parts of the world as a spice. It is also one of the most popular herbal remedies worldwide today. Garlic and its preparations have been widely recognized as agent for prevention and treatment of cardiovascular and other metabolic diseases, atherosclerosis, hypertension, thrombosis and diabetes. Allicin has been suggested as the potentially active component of garlic[7].

Animal studies suggested that garlic paste (3.8%), solvent-extracted fractions of garlic or garlic oil equivalent reduced the amount of serum cholesterol by 18 and 23% in broilers and 12 weeks-old Leghorn pullets, respectively[8]. Sklan *et al*.[9] observed depressed hepatic cholesterol concentration in chickens fed with 2% garlic while similar effects were found in rats[10]. Garlic extract fed to 5 weeks-old male broilers also exhibited hypocholesterolemic effects, mainly through the inhibition of the key enzymes[8]. Fresh garlic polysaccharide has been observed to exhibit immunomodulatory activities[11]. However, there is a paucity of information on the effect of inclusion of raw garlic in layers' diets on lipid metabolism and performance of laying hens. Therefore, the study was conducted to investigate the efficacy of raw garlic as performance booster and lipid-reducing agent in laying hens.

MATERIALS AND METHODS

The experimental protocol and feeding trial was carried out in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and as approved by the Animal Ethics Committee, Department of Animal Science, University of Ibadan, Nigeria. The study was conducted between January and April, 2015. The study adopted a completely randomized experimental design with six treatments. Treatment 1 was the control with 0% level of garlic while treatments 2-6 had 1, 2, 3, 4 and 5% inclusion level of raw garlic paste. The garlic used for the study was obtained from Sokoto, Northern part of Nigeria. The garlic was separated into cloves and ground into paste. The composition of the basal diet is shown in Table 1.

Management of experimental birds: Eighteen weeks old Isa Brown hens (n = 180) were randomly distributed to 6 dietary treatments. Each dietary treatment consisted of 6 replicates of 5 birds each. The birds were housed in galvanized iron cages under an intensive management system and the birds were allowed to acclimatize for 2 weeks and the study spanned over a 20 weeks period. Feed and water were given *ad libitum*.

Performance parameters measured: Feed consumption was obtained by finding the difference between the quantity of given feed/week and the left over. The body weight of each bird was taken at the start of the experiment. After which the body weights for each bird were measured every 4 weeks. The eggs laid were collected twice a day (i.e., morning and evening). The egg weights were measured daily. The percentage hen-day production was determined from the daily egg production records[12]:

$$\text{Hen-day production (\%)} = \frac{\text{Total Number of egg laid}}{\text{Number of birds} \times \text{Number of days}} \times 100$$

The egg mass was calculated as follows:

$$\text{Egg mass} = \frac{\text{Egg production} \times \text{egg weight}}{100}$$

Feed efficiency was measured thus:

$$\text{Feed efficiency} = \frac{\text{Feed intake (g)}}{\text{Egg mass}} \times 100$$

Table 1: Composition of diet fed to experimental birds

Ingredients	1 (0%)	2 (1% RG)	3 (2% RG)	4 (3% RG)	5 (4% RG)	6 (5% RG)
Maize	44.5	45.0	46.5	47.0	47.5	48.0
Palm kernel cake	9.0	9.0	8.0	8.0	8.0	8.0
Corn bran	8.0	8.0	7.0	6.0	5.0	4.0
Wheat bran	9.5	7.0	6.0	5.0	4.0	3.0
Groundnut cake	7.0	8.0	8.5	9.5	10.5	11.5
Soybean cake	9.5	9.5	9.5	9.0	8.5	8.0
Raw garlic	0.0	1.0	2.0	3.0	4.0	5.0
Fish meal	2.0	2.0	2.0	2.0	2.0	2.0
Bone meal	3.0	3.0	3.0	3.0	3.0	3.0
Oyster shell	6.6	6.6	6.6	6.6	6.6	6.6
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.15	0.15	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis						
Crude protein (%)	17.07	17.14	17.06	17.07	17.08	17.09
Metabolizable energy (kcal kg^{-1})	2600.00	2596.50	2596.10	2582.50	2568.90	2555.20

Eggs collected from each bird in each treatment at weeks 5, 10, 15 and 20 of the study were used for the measurements of internal and external egg quality parameters. The weight of eggs laid by the birds in each replicate was measured using a sensitive electronic scale. The weight of the shell was taken with a sensitive electronic scale. The egg shell thickness was taken using micrometer screw gauge. The egg yolk was separated from the albumen and the weight was also measured using an electronic scale. The yolk colour was determined using the colour range of Rouche colour fan numbered 1-15 while the yolk height was determined by the use of a vernier caliper. The vernier caliper was also used to measure the egg diameter.

Blood collection and analysis: Blood samples were collected from the birds at weeks 10 and 20 through the jugular vein. Blood samples for serum lipid analysis were collected in a sterile plain test tube, they were allowed to clot and then centrifuged at 2000 rpm for 10 min. The sera samples were carefully removed using pasture pipette and were stored at -20°C until further analysis.

Analysis of serum lipids: The serum samples were analyzed for total cholesterol (TC), triglycerides (Tg) and high-density lipoprotein cholesterol (HDL) using randox kit (CH201, TR210 and CH203, respectively, Randox Laboratories Limited, Antrim, UK). The LDL was calculated using Friedewald equation[13]:

$$LDL = TC - (HDL) - \frac{Tg}{5}$$

Collection of egg yolk: Eggs used for analysis of yolk cholesterol were collected from each bird at 10th and 20th weeks of the experiment. The yolk was carefully separated from the albumen using a separating pan. The samples were stored at -20°C until analysis.

Extraction and analysis of egg yolk cholesterol: One gram of yolk was placed into a centrifuge tube. Twenty milliliters of chloroform:methanol (2:1) was added, blended on a vortex mixture. A 10 L volume of the clear extract was used for the assay of total cholesterol. Cholesterol assay kit by Dialab (D00119, Dialab GmbH, Austria) was used for the cholesterol estimation.

Statistical analysis: Data were analyzed by one-way analysis of variance (ANOVA) using SPSS (Version 16.0. Armonk, NY: IBM Corp.)and the means were separated using Duncan's multiple range test of the same software. Values were expressed as mean (standard error of mean (SEM)). The level of statistical significance was $p<0.05$.

RESULTS

Effect of dietary inclusion of varying levels of raw garlic on the performance of laying hens is shown in Table 2. Birds fed 1 and 3% RG consumed slightly higher feed than birds fed the control diet, although the values were statistically similar. However, there was a significant decrease of 6.5% in the average daily feed intake of birds fed 5% RG compared with those fed with control diets. There was no significant difference in feed efficiency among all the treatment groups.

Table 2: Effect of dietary inclusion of varying levels of raw garlic on the performance of laying hens

	Treatments					
Parameters	Control diet	1% garlic	2% garlic	3% garlic	4% garlic	5% garlic
Average daily feed intake (g/bird)	99.70 (0.57)^{ab}	101.60 (0.45)^{a}	99.70 (1.06)^{ab}	101.60 (0.96)^{a}	98.10 (0.35)^{b}	93.20 (1.21)^{c}
Feed efficiency*	2.51 (0.208)	2.34 (0.123)	2.51 (0.150)	2.81 (0.148)	2.57 (0.061)	2.69 (0.257)
Average egg/weight (g)*	61.30 (1.31)	59.90 (1.18)	60.80 (0.86)	63.20 (0.94)	60.20 (1.03)	59.10 (1.12)
Hen day production (%)	66.90 (3.27)^{ab}	71.50 (1.98)^{bc}	77.40 (1.83)^{c}	70.70 (2.75)^{bc}	63.20 (2.43)^{a}	66.30 (1.81)^{ab}
Egg mass (g/day/hen)*	40.90 (3.56)	43.90 (1.95)	40.50 (3.01)	36.60 (2.34)	38.30 (0.82)	36.00 (3.52)

*Not significant, means with the same superscript in horizontal row are not significantly different ($p<0.05$)

Table 3: Effect of dietary inclusion of varying levels of raw garlic on the egg quality parameters

	Treatments					
Parameters	Control diet	1% garlic	2% garlic	3% garlic	4% garlic	5% garlic
Egg weight (g)	61.40^{ab}	59.90^{b}	60.80^{ab}	63.20^{a}	60.70^{ab}	59.10^{b}
Yolk weight (g)*	15.84	16.06	16.16	15.85	16.23	15.96
Shell weight (g)*	8.18	7.93	7.93	7.97	7.84	7.95
Shell thickness (mm)*	0.55	0.56	0.56	0.56	0.55	0.55
Yolk height	1.68^{b,c}	1.73^{a,b}	1.76^{a,b}	1.83^{a}	1.61^{c}	1 .73^{ab}
Egg length	5.89^{a,b}	5.87^{b}	5.90^{a,b}	6.01^{a-h}	6.03^{a}	5.97^{ab}

Means with the same superscript within the same row are not significantly different ($p>0.05$), *Non-significant

Table 4: Effect of dietary inclusion of varying levels of raw garlic on the serum lipids and egg cholesterol of laying hens

	Treatments					
Parameters	Control diet	1% garlic	2% garlic	3% garlic	4% garlic	5% garlic
Cholesterol	124.6 (1.77)^{a}	95.0 (1.33)^{b}	80.3 (0.92)^{c}	79.0 (2.75)^{c}	80.5 (2.51)^{c}	97.6 (1.51)^{b}
Triglycerides	81.3 (0.51)^{a}	78.3 (0.93)^{b}	69.9 (1.13)^{c}	67.8 (0.95)^{cd}	66.6 (0.93)^{d}	77.1 (0.99)^{b}
HDL-cholesterol	29.4 (3.69)^{a}	35.6 (0.83)^{b}	39.5 (1.26)^{c}	38.5 (1.45)^{c}	45.2 (1.87)^{d}	42.3 (0.88)^{e}
LDL-cholesterol	78.0 (2.54)^{c}	44.5 (2.50)^{b}	26.4 (1.14)^{a}	27.1 (2.38)^{a}	22.9 (2.23)^{a}	39.6 (2.91)^{b}
Egg yolk cholesterol (mg g^{-1})	14.4 (1.07)^{d}	12.2 (0.53)^{c}	11.3 (0.36)^{b}	11.0 (0.77)^{ab}	10.5 (0.56)^{a}	12.4 (0.61)^{c}

Means with the same superscript in horizontal row are not significantly different ($p>0.05$)

Birds fed with 2% RG had a significant ($p<0.05$) higher value of hen day production (HDP) compared to birds fed with the control diet, 4 and 5% RG. The lowest HDP was observed in birds fed 4% RG although this value was not significantly different from the HDP values of birds fed with the control diets. There was no significant effect of the dietary treatments on the values for egg mass as averaged over the experimental period. Table 3 shows the effect of dietary inclusion of varying levels of raw garlic on the egg quality parameters. Intake of varying levels of raw garlic by laying hens did not have any significant effect on yolk weight, shell weight and shell thickness. The values obtained for egg weight were statistically similar across the treatments. The highest egg yolk height was recorded in bird fed 3% RG. This value was significantly ($p<0.05$) higher than the egg yolk height of birds fed with the control diet and 4% RG. The values recorded for yolk height of laying chickens fed 1, 2 and 5% RG were similar. The highest mean egg length was observed in chickens fed with 4% RG. Although the mean egg length of hen fed 4% RG was similar to the mean egg length of chickens fed control diet, 2, 3 and 5% RG. The birds fed 1% RG had the lowest mean egg length.

The effect of dietary inclusion of varying levels of raw garlic on the serum lipids and egg cholesterol of laying hens is presented in Table 4. The highest hypocholesterolemic effect of RG was observed in birds fed 3% RG (79 mg dL^{-1}), followed by birds fed with 2, 4, 1 and 5% RG, respectively. The effect of inclusion of RG in the diets of laying on TC was similar for birds fed with 2, 3 and 4% RG. However, birds fed with 2, 3 and 4% RG had significantly ($p<0.05$) higher value compared with birds fed with 1 and 5% RG. Birds fed control diet had the highest TC. The dietary treatments significantly ($p<0.05$) influenced serum triglycerides of laying hens. Birds fed with 1, 2, 3, 4 and 5% RG had significantly ($p<0.05$) lower Tg relative to the Tg of birds fed with the control diet. The mean values of Tg for birds fed with 2 and 3% RG were similar. The lowest Tg was observed in birds fed with 4% RG although, this value was similar to the Tg of birds fed with 3%. However, the Tg of birds fed with 3 and 4% RG were significantly ($p<0.05$) lower than the Tg of birds fed with 1 and 5% RG. The value of Tg of birds fed with 1 and 5% RG were not significantly different.

Inclusion of graded level of garlic in layers' diets significantly ($p<0.05$) elevated the serum HDL concentration of laying hens in comparison with the HDL of birds fed with

the control diet. Birds fed 4% RG recorded highest HDL concentration of 45.2 mg dL^{-1} and this value was significantly ($p<0.05$) higher than the HDL of birds fed with other experimental diets. Although there was no significant difference in the values of HDL of birds fed 2 and 3% RG, these values were significantly ($p<0.05$) higher than the HDL concentration of birds fed with 1% RG and those fed with the control diet. There was an increase of 21.1, 34.4 31.0, 53.7 and 43.9% in HDL concentration of birds fed 1, 2, 3, 4 and 5% RG respectively when compared with the HDL of birds fed with the control diet.

A significant ($p<0.05$) reduction in the values of LDL was observed in birds fed with graded levels of RG when compared with LDL of birds fed with the control diets. The lowest LDL was recorded in birds fed 4% RG although this value was not significantly different from LDL of layers fed with 2 and 3% RG. The LDL of layers fed with 1 and 5% were similar but were significantly ($p<0.05$) higher than LDL of bird fed with 2, 3 and 4% RG.

A general decline of the mean egg yolk cholesterol was observed as the level RG increased up to 4% after which there was a decline of 13.4% when laying hens were fed with 5% RG relative to egg yolk cholesterol of birds fed with the control diet. There was no significant difference between egg yolk cholesterol of birds fed 1 and 5% RG, although these values were significantly ($p<0.05$) lower than the egg yolk of birds fed with the control diet. The egg yolk cholesterol of birds fed with 2, 3 and 4% were similar.

DISCUSSION

The low feed intake of birds fed 5% RG could be attributed to high inclusion of allicin in the diet which probably made the diet to be less acceptable to birds. Allicin is assumed to be the potential active component of garlic and increasing its concentration in diets may affect the palatability of such diets in birds[14]. Cullen *et al.*[15] also reported a reduction in feed intake of pigs fed 1 and 10 g kg^{-1} garlic diet during the grower phase. The effect of inclusion of RG at varying levels in layers diets on feed intake observed in this study is in agreement with Olobatoke and Mulugeta[16] who recorded a decrease in feed consumption when 5% garlic powder was included in the diet of 30 weeks-old Dekalb white strain hens.

The non-significant difference observed among the treatment groups for feed efficiency in this study shows that the inclusion of raw garlic in layers' diets up to 5% made no apparent difference in the diets that could influence the feed conversion ability of the birds. Chowdhury *et al.*[17] earlier reported that inclusion of garlic pastes up to 10% did not have significant effect on feed efficiency.

The highest egg weight recorded for birds fed 3% RG may be said to have a positive effect on the protein and energy utilization of the birds. Yalcin *et al.*[18-19] reported that 5 and 10 g kg^{-1} of garlic powder resulted in increased egg weights of laying quails. However, garlic products did not have significant effect on egg weight in other studies[17,20-21]. The variations observed may be due to the use of different commercial garlic products and the preparation methods of garlic powder. The mean egg weights for all the treatment groups were above 58 g reported by Oluyemi and Roberts[22] but were similar to that reported by Ao *et al.*[23] and Mohebbifar and Torki[24].

The inclusion of 2% RG in layers' diet resulted in the highest value for HDP, which suggests that 2% level of RG may be a better level of inclusion than 0, 4 and 5% RG in improving egg production of Isa Brown layers. This observation is in line with the findings of Rahardja *et al.*[25] who reported significant improvement in egg production of 27 weeks Hysex Brown hens fed with 2% garlic powder. In contrast, Yalcin *et al.*[18] reported that supplementation of layers' diet with 1, 5 and 10 g kg^{-1} garlic powder had no effect on egg production, which may be due to the use of very low levels of garlic powder in the diet. Similar findings as observed for egg mass in the present study were reported by Khan *et al.*[26] for 0, 2, 6 and 8% garlic powder on 30 weeks-old Desi layers.

The result of this study revealed that RG did not result in physiological changes leading to the formation of egg yolk in laying hens. The results of this study agree with the findings of the previous studies[18,27]. However, Mottaghitalab and Taraz[28] showed that the inclusion of 5, 10 and 15 g kg^{-1} garlic powder decreased egg yolk weight. Chowdhury *et al.*[17] reported that egg yolk weight responded quadratically in weeks 3 and 4 with increasing levels of sun-dried garlic paste.

Inclusion of RG in the diets of laying chickens had no significant effect on egg shell weight and thickness of the birds, which probably suggests that RG did not affect the calcium to phosphorus ratio and metabolism in the layers' diets as the level of calcium and phosphorus are important in layers diet for the formation of egg shell. The results of this present study corroborate the findings of the previous research work by Khan *et al.*[21] who observed no significant difference among treatment groups when white Leghorn layers were fed 0, 2, 6, 8% garlic powder. Lim *et al.*[20] earlier reported no significant changes in egg shell thickness when

Table 5: Linear regression of serum lipids and egg cholesterol on dietary raw garlic in laying hens

Parameters	Constant	Garlic (r = 0.80)	t-value	p-value	R^2	F-value	p-value
Total cholesterol	110.8 (15.26)	-5.10 (3.91)	-1.31	0.260	0.30	1.72	0.2601
Triglycerides	79.3 (5.54)	-1.66 (1.42)	-1.17	0.307	0.25	1.37	0.3071
LDL-cholesterol	65.3 (15.92)	-7.30 (4.09)	-1.79	0.148	0.44	3.20	0.1481
HDL-cholesterol	29.2 (2.54)	2.63 (0.65)	4.04	0.016	0.80	16.33	0.0156
Yolk-cholesterol	13.5 (1.17)	-0.44 (0.30)	-1.47	0.217	0.35	2.15	0.2165

Hyline Brown layers were fed 0, 1, 3 and 5% garlic powder for 5 weeks. There was no mortality during the experimental period. Yalcin *et al.*[18] also recorded no mortality upon feeding of layers with 0, 5 and 10 g kg^{-1} garlic powder over a 22 weeks experimental period.

The RG reduced serum TC in the experimental birds which confirmed the hypocholesterolemic property of garlic as earlier reported[21]. In a related experiment, plasma cholesterol was reduced by 30% when rats were fed diets supplemented with 2 or 3% garlic powder[10]. The level of reduction of serum TC in this present study is higher than that reported by Khan *et al.*[21] who recorded 1.7% reduction in serum TC when laying hens were fed 8% garlic powder. The difference in the level of reduction could be due to different preparations of garlic. However, Qureshi *et al.*[29] reported that the equivalent of 1, 2, 4, 6 and 8% garlic paste reduced serum TC by 18, 21, 21, 24 and 25%, respectively, in male broiler chickens. Similarly, in another study, Qureshi *et al.*[8] also reported that serum cholesterol of White Leghorn pullets was reduced by 20-25% with supplementation of garlic paste, a solvent-extracted garlic pastes and commercial garlic oil. Such reduction with supplementation of garlic may be due to the inhibition of fatty acid synthesis as organic tellurium compounds and allicin found in garlic might contribute to lower serum cholesterol by inhibiting squalene epoxidase which is needed in the synthetic pathway of cholesterol. It may also be due reduction in major regulatory enzyme of cholesterol biosynthesis activity[29-30]. Sklan *et al.*[9] also reported that the inclusion of 2% lyopholized garlic in chick diets depressed hepatic cholesterol levels in chicks, suggesting that feeding of garlic may down-regulate liver cholesterol synthesis.

The observation in this study shows that serum Tg was significantly reduced by graded levels of RG in layers' diet. This result corroborates the observations of Yeh and Liu[31] reported 30% reduction in triacylglycerol when 2 g/100 g of age garlic extract was supplemented in Sprague-Dawley rats' diet. The study also showed that the triacylglycerol-lowering effect of garlic might be explained in part by its inhibiting action on fatty acid synthesis. However, this present study recorded a higher decrease of 36.6% in serum Tg concentration at 3% inclusion level of RG fed to laying hens.

Although the effect of the inclusion of RG at 3% on serum Tg did not show any significant difference in serum Tg when compared to layers fed 4% RG. This indicate that inclusion of 3% RG in the diet of laying hens might be enough to reduce the serum Tg maximally. On the contrary, Horton *et al.*[32] and Ghasemi *et al.*[33] reported that Tg was not affected by garlic or a mixture of garlic and thyme. The low inclusion level of this herbal mixture could be responsible for its non-significant effect on Tg compared with this present study.

The increasing effect of garlic on serum HDL concentration is advantageous, because serum HDL inhibits oxidation of serum LDL and thus prevents formation of foam cells by promoting cholesterol efflux in the intima[34]. It was observed in this current study that the values of HDL concentration increased linearly in response to increasing levels of dietary garlic (r = 0.80) (Table 5). In addition, an inverse relationship could be deduced from the values for serum cholesterol and HDL concentration. As the value of serum cholesterol decreased with increasing level of RG included in the layer's diet, the HDL concentration increased up to 4% RG inclusion. This observation suggests a direct relationship between the factors that could limit the production of serum TC and HDL concentration in laying hens. In general, the HDL concentrations tended to follow an inverse pattern from other lipids. This results also corroborates the findings of Aouadi *et al.*[35], who recorded a significant increase in the value of HDL when rats were fed 10% fresh garlic. However, Canogullari *et al.*[27] did not observe any difference in the values of HDL when 0, 0, 5, 1 and 2% garlic powder were fed to layers, the effect which may be due to inclusion of lower doses of garlic.

The hypocholesterolemic effect of garlic was exhibited in layers fed 1, 2, 3, 4 and 5% RG. The present study reflected that the LDL concentration decreased with increasing level of RG in the layers' diet. Similar results were observed by previous researchers[7,29,36].

The reduction of the egg yolk cholesterol observed in this study tended to follow the pattern of reduction of LDL-cholesterol. All the laying hens fed RG had significantly lower egg yolk cholesterol compared with birds fed control diet. Qureshi *et al.*[29] reported that the suppressive action of garlic could be evident at level of cholesterol biosynthesis, in that garlic could suppress HMG-CoA reductase, the effect of which could manifest in the reduction of LDL-cholesterol. Previous studies also reported that garlic resulted in a

reduction in yolk cholesterol[18]. However, at 0.5% inclusion of garlic powder in layers diet did not exert reducing effect on egg yolk cholesterol[18]. The reduction of yolk cholesterol recorded in this study compared with the previous research works[18,29] may be attributed to inclusion of high dosage of RG in this present study. Also, the contrast in the level of reduction of yolk cholesterol due to intake of garlic by laying hens may be associated with differences in the age and or strains of the experimental hens.

CONCLUSION AND FUTURE RECOMMENDATION

Inclusion of raw garlic had no effect on feed efficiency, average egg weight, egg mass, yolk weight, shell weight and shell thickens. Five percent RG inclusion resulted in reduced feed intake in the experimental laying hens while 2% inclusion of RG had the highest hen-house production.

Specific conclusion about the use of raw garlic as growth promoter for laying hens may not be ascertained through the findings of this study, further studies are therefore recommended to ascertain that.

SIGNIFICANCE STATEMENTS

Raw garlic at the inclusion levels improved high-density lipoprotein cholesterol but reduced total cholesterol, yolk cholesterol and low-density lipoprotein cholesterol contents in the experimental animals. This study further confirms the hypocholesterolemic effect of garlic and its use in animal and human nutrition for health improvement.

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