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THE EFFECT OF GARLIC (ALLIUM SATIVUM) ON LIPID PROFILE IN RABBITS

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ABSTRACT

This study was conducted to investigate the cholesterol-lowering property of garlic (*Allium Sativum*) in whole blood of egg yolk induced hypercholesterolemia in rabbits. Forty rabbits of both sexes of 13.1 ± 28.4 weeks of age with average body weights of 1251.9 ± 512.2g were used for the experiment. The animals were divided into eight groups comprising control and seven experimental groups with 5 rabbits per group. The animals were acclimatized with grower's mash for one week after which the control group was fed with grower's mash and the seven experimental groups were fed with grower's mash supplemented with 10% egg yolk, 20% egg yolk, 2% garlic, 4% garlic, 10% egg yolk + 2% garlic, 20% egg yolk + 2% garlic, and 20% egg yolk + 4% garlic respectively for five weeks. Animals were phlebotomized through prominent ear veins and blood samples (2 ml) were collected from rabbits in each group before and after the treatment (diet administration) to assay for total

cholesterol (TC), HDL-cholesterol and triglycerides (TG) using the CardioChek® analyzer; the LDL-cholesterol was determined using Friedewald formula. The TC analysis shows that there was no significant difference between the control and the treatment groups (P>0.05). The HDL-Cholesterol analysis indicates no significant difference between the control and the treatment groups (P>0.05) except the group that received 10% egg yolk + 2% garlic supplementation (P<0.05). The LDL-Cholesterol analysis show significant differences exist between the control and all other treatment groups (P<0.05) except the group that received 2% garlic supplement, where a decrease (P>0.05) was observed. The results of TG analysis show no significant difference between the control and the treatment groups that received 10% egg yolk, 2% garlic or 10% egg yolk + 2% garlic supplementations (P>0.05). However, there was significant increase (P<0.05) in the TGs of the treatment groups that received 20% egg yolk, 4% garlic, 20% egg yolk + 2% garlic or 20% egg yolk + 4% garlic compared to the TG of the control group. While egg yolk supplementation did not induce hypercholesterolemia; it was observed that garlic powder supplementation did not demonstrate significant hypocholesterolemic effect on the lipid profile of rabbits.

Key words: Garlic (*Allium sativum*); Cholesterol; Grower's mash;

BACKGROUND OF STUDY

(*Heading 1)*

Cardiovascular diseases are the principal causes of mortality in middle aged and older people worldwide [1]. Coronary heart disease (CHD) is the most common cardiovascular disease, and atherosclerosis is considered a major clinical factor. Clinical and experimental evidences suggest that increased circulatory concentrations of serum cholesterol are

associated with atherosclerosis [2]. Reference [3] have demonstrated that high dietary concentration of cholesterol is linked to increased ratio of total cholesterol to high-density lipoprotein cholesterol (HDL-C) in human and increased ratio of low-density lipoprotein cholesterol (LDL-C) to high-density lipoprotein cholesterol (HDL-C) [4]. The concentration of LDL-C present in circulation is a marker for investigating suspected CHD.

Consumption of eggs and egg baked food products is a common choice of the urban population in developed and developing countries. In the course of this research work, it was suggested that egg yolk contain a high concentration of cholesterol; this diet has the potential to increase the ratio of total cholesterol (TC) to HDL-C and increase the ratio of LDL-C to HDL-C in human [4]. The LDL particle being a carrier of large proportion of lipids (distributes cholesterol to peripheral tissues) in the blood, hence its low density in aqueous media is associated with re-uptake via receptor-mediated endocytosis [3]. A defective or deficient or repressed LDL-receptor system result to an abnormally high circulatory LDL-C concentration in the blood. In normal physiological state, dietary cholesterol is distributed to peripheral tissues and transported to the liver as chylomicron remnant which represses the synthesis of LDL-receptor, favors liver mobilization of VLDL (LDL) and thereby serves as product inhibition to the rate-limiting step enzyme “Hydroxyl-Methyl-Glutamyl-CoA (HMG-CoA) reductase” in the liver [5]. A diminishing circulatory concentration of LDL-C stimulates the synthesis of more LDL-receptor that activates an endocytotic receptor-mediated uptake mechanism.

Garlic (*Allium Sativum*) belongs to the family of Alliaceae [6]; one of the oldest cultivated plants used as food with applicable health benefits. Some reported uses to which it has been applied include carcinogenicity, CHD, obesity,

hypercholesterolemia, hypertension and others [7]. In this study, the effect of graded levels of egg yolk on the whole blood lipid profile of fed rabbits was investigated, the effect of garlic powder supplemental diet on egg yolk-induced hypercholesterolemia and the effect of a combination of egg yolk and garlic powder supplement on the whole blood lipid profile of fed rabbits were investigated. The aim of this research study was to investigate and provide a rationale for the application of garlic in hypercholesterolemia.

Objective of Study: is to practically demonstrate induced hypercholesterolemia with egg-yolk rich formulated diet while attenuating hypercholesterolemia with garlic (*A*. sativum) powder.

STUDY PROTOCOL (*Heading 2)*

Materials and Methods

The study on the Cholesterol-lowering effect of garlic (*Allium Sativum*) was conducted in the animal house and laboratory of the Department of Biochemistry, Ambrose Alli University, Ekpoma. Nigeria. **Design:** Forty rabbits of 13.1 ± 28.4 weeks of age with average live body weight of 1251.9 ± 512.2 g were purchased from Danlami farms in Jos South local Government Area, Plateau State (Northern middle belt of Nigeria). All animals were divided into eight groups comprising control and 7 experimental groups with 5 rabbits per group. The experimental animals were housed in compartments; at a temperature of 32 ± 7˚C. The animals were acclimatized for one week before the commencement of the experimental period during which the animals received only growers' mash and water *ad Libitum*. The control group was fed with grower's mash (table 2) and the experimental groups with egg yolk, garlic, and garlic/egg yolk formulated diet. Egg yolk was employed as source to induce cholesterol in this study. Blood samples were collected from forty animals into an ethylenediaminetetraacetate (EDTA) container at the beginning (week 0) and the end (week 5) of the experimental study to determine changes in selected lipid

parameters. Egg yolk was employed as source of cholesterol in this study. Egg was hard-cooked and egg white removed to obtain the yolk; the egg yolk was crushed and sundried. Garlic was purchased from the market, its bulbets were separated, cut into smaller pieces and then sundried for seven to eight days. After sun-drying, the garlic was milled into powder. **Collection of blood sample**: venous blood samples were collected from the ear lobe of the rabbits prior to administration of treatment feed (week 0) and at the end of the experimental study (week 5) and stored in EDTA containers to determine some selected lipid parameters. **Determination of Egg yolk Lipid:** A 0.1g of egg yolk sample was weighed and homogenized in 15 ml chloroform-methanol (2:1). The method described by [8] was adopted to obtain a 99% lipid extract required for the analyses of egg yolk cholesterol and triacylglycerol. Total cholesterol was determined by the enzymatic cholesterol-oxidase method [9], Triacylglycerol (TG) was determined by Glycerol-3-Phosphate Oxidase-Peroxidase-4-aminophenazone (GPO-PAP) method [10]. High-density Lipoprotein cholesterol (HDL-C) was determined by Phosphostungstate-Mg^{2+} precipitation method and the Low-density Lipoprotein cholesterol (LDL-C) was estimated by the method of Friedewald formula [11]. **Determination of Lipid profile in whole Blood:** Venous blood samples was collected by pricking the prominent veins of the earlobe of the animal subjects with lancet supplied by Polymer Technology System (PTS manufactured in Ohio, U.S.A) before and after the study. Whole blood samples were analyzed for Total Cholesterol, Total Triglycerides and HDL-Cholesterols using the test strips provided by CardioChek Brand Analyzer PTS and LDL-Cholesterol was calculated using the Friedewald formula [11]. CardioChek is a portable test system that uses whole blood to analyze selected lipid parameters. The CardioChek analyzer test strips come with an electronically calibrated MeMo Chip that contains the test name, lot number and test strip expiration date. **Statistical Analyses:** The significant differences was tested by analyses of variance and Tukey-Kramer test using the GraphPad InStat version 2.04a (GraphPad Software Inc, San Diego, California, USA, www.graphpad.com) in this experimental study. The student's t-test distribution was used to compare test results obtained at the start of the experiment (week 0) with the final (week 5) results which include the control group results.

Table 1. Composition of Grower's Mash

Components	Quantity
Crude Protein (%)	16.00
Fats and oil (%)	5.00
Crude Fibre (%)	7.00
Calcium (%)	1.00
Phosphorus (%)	0.45
Lysine (%)	0.75
Methionine (%)	0.36
Salt (min) (%)	0.30
Kcal/kg	2450
Metabolizable energy (min)	

Source: TOPFEEDS (2011)
Net Weight (25kg).

Table 2: Diet Composition of Experimental groups (g/150g)

	Experimental Groups							
	Control	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**
Growers Mash (g)	150	135	120	147	144	132	117	114
Egg Yolk (g)	-	15	30	-	-	15	30	30
Garlic Powder (g)	-	-	-	3	6	3	3	6
Percentage egg yolk/garlic (%)	-	10	20	2	4	12	22	24
Total Cholesterol (mg/g)*	-	4000.1	8000.1	-	-	4000.1	8000.1	8000.1

*Cholesterol concentration of formulated diet was calculated from the mean total cholesterol of egg yolk (266.67 ± 33.33)

RESULTS OF FINDINGS

DETERMINATION OF EGG YOLK LIPID PROFILE

Some selected lipid parameters were determined for the egg yolk that was used in this study as source of cholesterol. The following results were obtained as shown in table 3.

Table 3. Results of analyzed lipid parameters in egg yolk

Total Cholesterol (mg/g)	Triglycerides (mg/g)	HDL-Cholesterol (mg/g)	LDL-Cholesterol (mg/g)
266.67 ± 33.33	80.00 ± 23.09	70.00 ± 0.00	180.67 ± 29.42

Data report are Mean ± SEM.

The Effect of Egg yolk supplemental diet on whole blood lipid parameters in rabbits.

The changes in some selected lipid parameters in whole blood of rabbits that received graded levels of egg yolk (10% and 20%) and control (no egg yolk and garlic supplementation) were determined as shown in figures 1, 2 and 3. After 5 weeks of grower's mash administration to control group, the results of statistical analysis show 15.6% increase and 30.4% decrease ($P<0.05$) in Low-density Lipoprotein(LDL) cholesterol and Triglycerides (TG) respectively; there was 21.4% decrease in High-density Lipoprotein (HDL) cholesterol. The feed (grower's mash) however had no significant effect on Total cholesterol (TC). The rabbits fed 10% and 20% egg yolk supplementation resulted in 48.7% and 13.2% increase ($P>0.05$) respectively in whole blood HDL-Cs; 26.8% and 2.8% reduction in whole blood LDL-C ($P>0.05$) respectively. There was 13.5% ($P<0.05$) and 11.3% ($P>0.05$) decreases in the TGs for rabbits fed 10% and 20% egg yolk supplemented diets respectively. The 10% and 20% egg yolk supplementation was however observed to show no effect on whole blood TC in fed rabbits after 5 weeks of feed administration as presented in table 4.8. The results of LDL-C for rabbits fed 10% and 20% egg yolk supplementation indicate non elevated LDL-C, instead, reduced the LDL-C concentration were recorded.

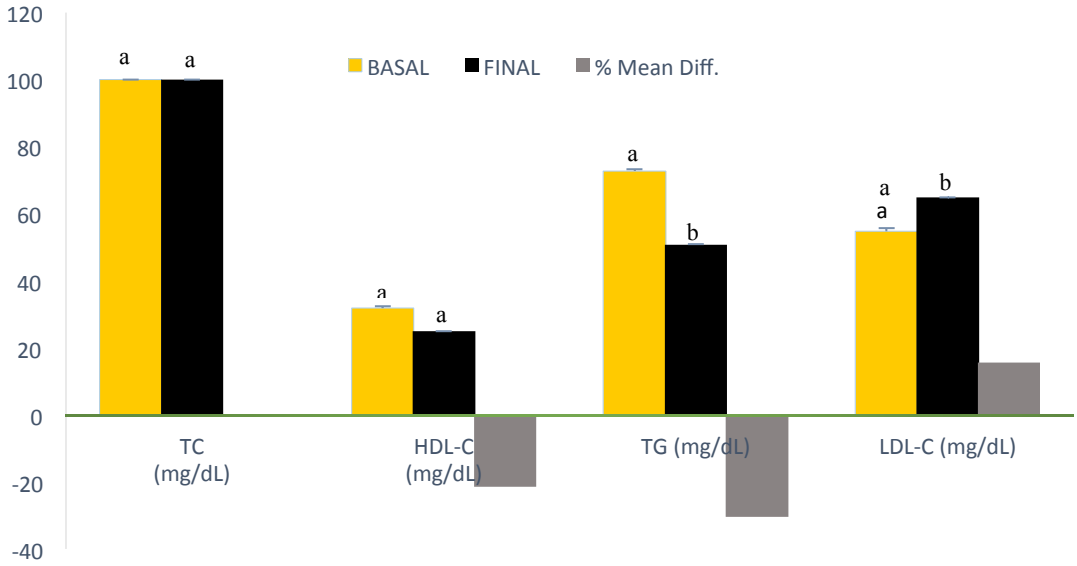

Fig 1. Control group: 0 % egg yolk, 0% garlic in 150 g grower's mash

Data report are Mean ± SEM (n=5). Mean with different letters differ significantly ($P < 0.05$). Mean % difference after 5 weeks of treatment and negative result indicates reduction.

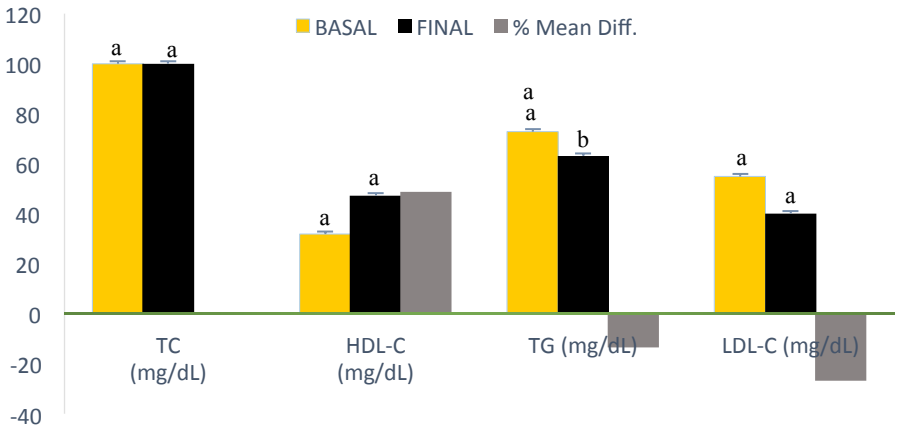

Fig 2. 10 % egg yolk in 150 g grower's mash

Data report are Mean ± SEM (n=5). Mean with different letters differ significantly ($P < 0.05$). Mean % difference after 5 weeks of treatment and negative result indicates reduction.

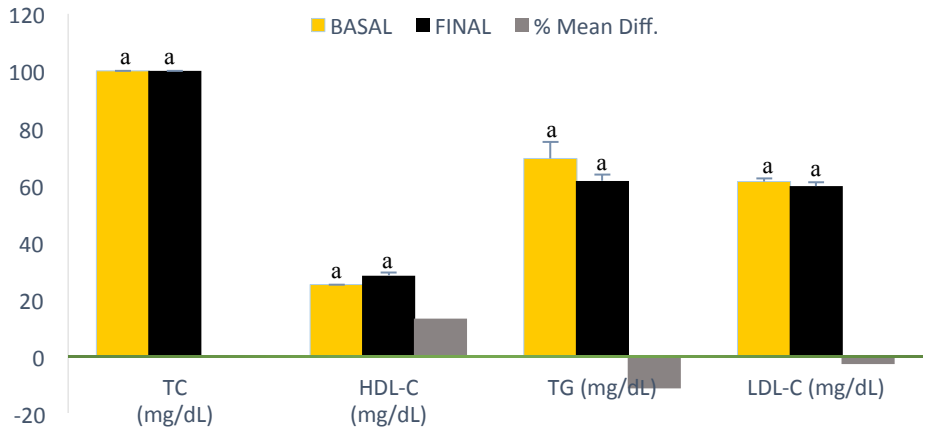

Fig 3. 20 % egg yolk in 150 g grower's mash

Data report are Mean ± SEM (n=5). Mean with different letters differ significantly ($P < 0.05$). Mean % difference after 5 weeks of treatment and negative result indicates reduction.

DISCUSSION

The Effect of Egg yolk supplemental diet on whole blood lipid parameters in rabbits

The results obtained from the analyses of whole blood lipid parameters of rabbits fed grower's mash (control) and rabbits fed graded levels of egg yolk are presented in figures 1, 2 and 3. The TC in the present study was observed to be unaffected in the control and the animals that received supplemental graded levels of egg yolk. This finding is in line with the report of [12]. Reference [13] have reported increased HDL-C of subjects fed 3 or 6 egg per day in addition to their habitual diets for 3 months; this finding is similar to the HDL-C of the present study. Although, we used egg yolk in this study, whole egg was used by Oh and Miller. The reduction in LDL-C ($P>0.05$) and TG ($P<0.05$) observed in rabbits fed 10% and 20% egg yolk supplementation (figure 2 and 3) is similar to the report observed in hyperlipidemic adults fed 2 or 3 eggs per day for 6 weeks [14]. Contrary completely to the present study is the report of [12]. Reference [12] reported that serum TC, HDL, LDL-Cs and TG were unaffected by consumption of 1 egg per day in 5 weeks. The difference observed in the analysis of lipid parameters in the present study compared to the reports of [12] could be attributed to the difference in egg preparation, quantity of egg yolk used or probably difference in sample used for lipid assessment. While 10 or 20% egg yolk supplemental diet per day was fed to rabbits in 5 weeks in the present study, one whole egg per day was fed to

human subjects. The present study used whole blood for lipid profile assessment of fed rabbits while [12] used serum (sample).

The Effect of garlic (*Allium sativum*) supplemental diet on whole blood lipid parameters in rabbits.

The results of some selected lipid profile determined for rabbits that received graded levels of garlic (2% and 4%) are presented in figures 4 and 5. 2% garlic supplementation did not show significant effect on whole blood TC and HDL-C, but rabbits fed grower's mash and 4% garlic supplementation resulted in 21.4% ($P>0.05$) reduction in HDL-Cs after 5 weeks of feed administration; however had no significant effect on TC as in the case of 2% garlic supplementation. The 2% and 4% garlic test diet resulted in 5.3% ($P<0.05$) and 18.2% ($P>0.05$) increases in LDL-C respectively and the TGs of rabbits fed 2% and 4% garlic supplementation decreased by 20.8% ($P<0.05$) and 29.9% ($P>0.05$) respectively after 5 weeks of feed administration as determined in the control group fed grower's mash.

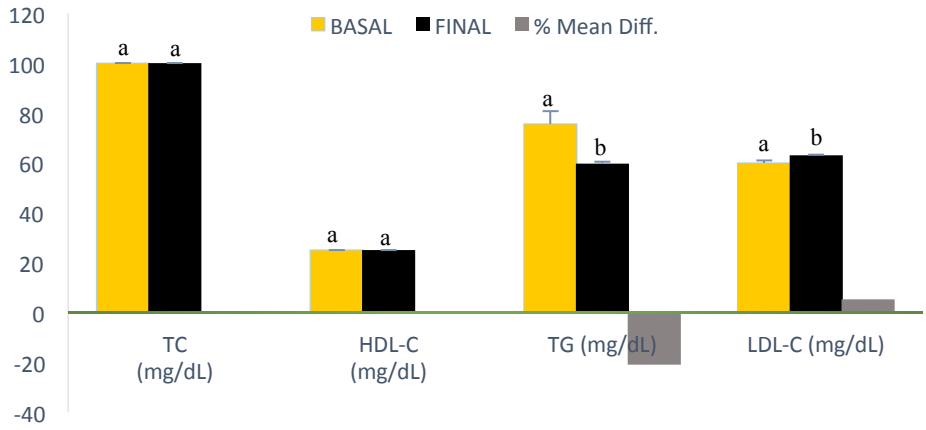

Fig 4. 2 % garlic in 150 g grower's mash

Data report are Mean ± SEM (n=5). Mean with different letters differ significantly ($P < 0.05$). Mean % difference after 5 weeks of treatment and negative result indicates reduction.

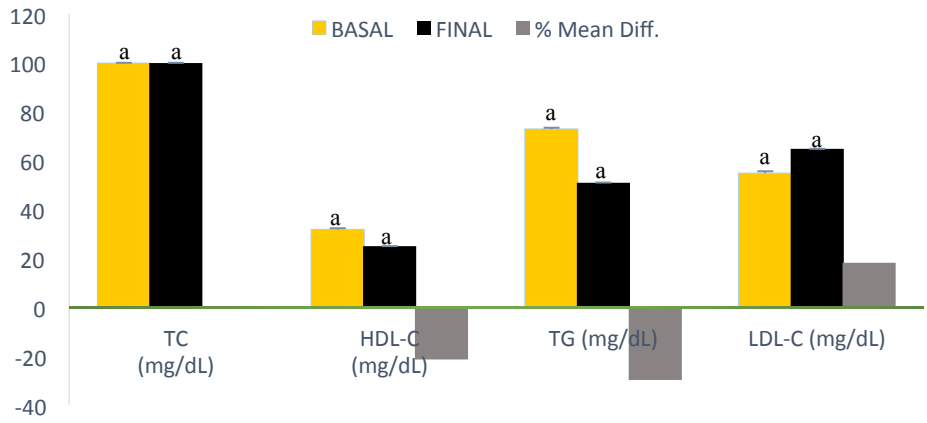

Fig 5. 4 % garlic in 150 g grower's mash

Data report are Mean ± SEM (n=5). Mean with different letters differ significantly ($P < 0.05$). Mean % difference after 5 weeks of treatment and negative result indicates reduction.

DISCUSSION

The increased and decreased LDL-C and TG respectively observed in rabbits fed 2% or 4% garlic supplementation (figures 4 and 5) is similar to the study reported by [15]. Reference [15] reported that quails fed graded levels of garlic powder (1, 2 or 4%) in 12 weeks increased serum LDL-C and reduced TG. Although, Reference [15] used serum while the present study used whole blood for the determination of the selected lipid parameters, however the same similarities in assessed lipid profile was observed. Reference [16] have also reported that hens fed 0.5, 1 or 2% garlic powder did not influence and significantly affect serum HDL-Cholesterol, although it reduced it. This finding is in line with the result observed for HDL-C (fig.5) in the present study. Reference [17] have reported that chemically well characterized garlic preparation (2.1g/day) had no effect on the plasma TC of overweight and smoking subjects. The finding of [17] is in line with the present study that reported that graded garlic supplementation did not affect total cholesterol. In contrast, [18] have reported that a day old chick fed graded levels of garlic powder (1, 3 and 5%) for 35 days resulted in decrease in serum TC ($P<0.05$), increase in HDL-C ($P<0.05$) and decrease in LDL-C ($P<0.05$). However, [18] did not report the effect of garlic powder supplementation on serum TG. The investigation conducted by [19] have reported that garlic therapy (300mg three times daily of kwai garlic tablets, Lichtwer Pharma, Pittsburgh, Pennsylvania) administered to human subjects for 12 weeks had no effect on plasma major lipoproteins and

total triglycerides. The report of [19] completely contrasts the result of lipid profile in the present study. The difference in quantity of garlic dose and experimental subjects could be responsible for the difference in lipid profile result. The present study used 20 or 40mg garlic powder per gram of grower's mash per day of garlic supplementation, whole blood and rabbit models while [19] used 900mg garlet per day (garlic tablets), plasma and human subjects.

The Effect of egg yolk-garlic (*Allium sativum*) supplemental diet on whole blood lipid parameters in rabbits.

The results of some selected lipid parameters of Rabbits fed graded levels of egg yolk mixed with garlic powder (10% egg yolk + 2% garlic, 20% egg yolk + 2% garlic and 20% egg yolk + 4%) supplemental diets resulted in increased TC (37.2%, 14.5% and 28.8%, $P>0.05$) respectively after 5 weeks of test diet administration (Figs 6, 7 and 8). The TC of the control group (no egg yolk or garlic powder) was however unaffected by grower's mash. The increases in TC of rabbits fed test diets however did not follow any pattern of increase. The HDL-C of rabbits fed 10% egg yolk + 2% garlic increased (50.5%, $P<0.05$) while the rabbits fed 20% egg yolk + 2% garlic and 20% egg yolk + 4% garlic had their HDL-C non-significantly increased (29.6% and 42.7%) respectively, the HDL-C of rabbits fed grower's mash (control) was reduced (27.2%, $P>0.05$). The LDL-C of 34.9% was recorded by rabbits fed 10% egg + 2% garlic; the LDL-Cs (10.9% and 7.5%) of the rabbits that received 20% egg yolk + 2% garlic and 20% egg yolk + 4% garlic respectively were reduced compared to the LDL-C of rabbits that received 10% egg yolk + 2% garlic. Although, there was increase in LDL-C in the control group and it is also considered reduced compared to the elevated LDL-C of the rabbits fed 10% egg yolk + 2% garlic. The rabbits fed 10% + 2% garlic recorded the highest LDL-C (34.9%) while rabbits fed with 20% egg yolk + 4% garlic supplementation recorded the lowest LDL-C. The TGs of the rabbits fed grower's mash and 10% egg yolk + 2% garlic were significantly reduced. While the TGs of the rabbits fed 20% egg + 2% garlic and 20% egg yolk + 4% garlic were reduced non-significantly. Rabbits fed 10% egg yolk + 2% garlic recorded the highest ($P<0.05$) reduction in TG (34.1%) and the lowest non-significant reduction (8.0%) was recorded in rabbits that received 20% egg yolk + 2%garlic. The results presented in the table 4.10 indicate elevated LDL-C in the control and rabbits fed graded levels of egg yolk and garlic supplementation and the combination of garlic and egg yolk supplementation did not reduce the elevated LDL-C.

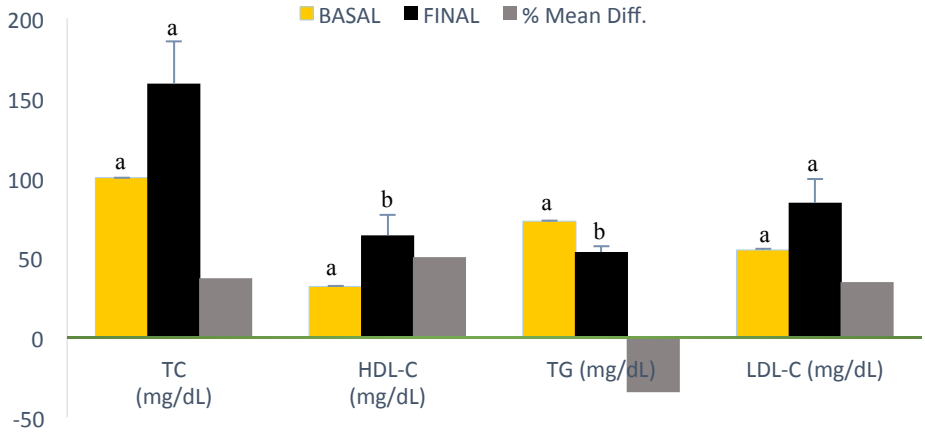

Fig 6. 10 % egg yolk + 2 % garlic in 150 g grower's mash

Data report are Mean ± SEM (n=5). Mean with different letters differ significantly ($P < 0.05$). Mean % difference after 5 weeks of treatment and negative result indicates reduction.

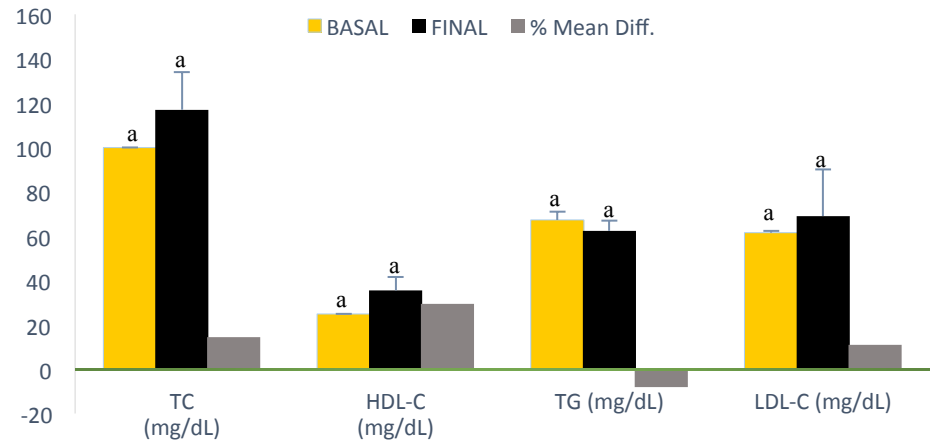

Fig 7. 20 % egg yolk + 2 % garlic in 150 g grower's mash

Data report are Mean ± SEM (n=5). Mean with different letters differ significantly ($P < 0.05$). Mean % difference after 5 weeks of treatment and negative result indicates reduction.

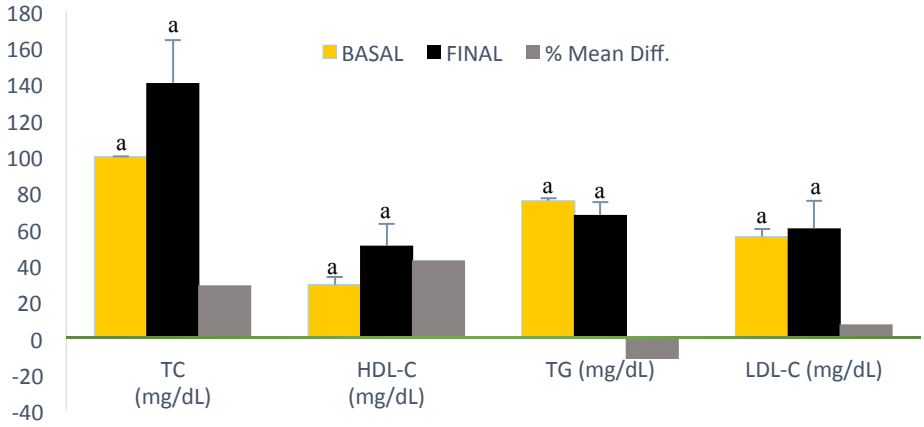

Fig 8. 20 % egg yolk + 4 % garlic in 150 g grower's mash

Data report are Mean ± SEM (n=5). Mean with different letters differ significantly ($P < 0.05$). Mean % difference after 5 weeks of treatment and negative result indicates reduction.

DISCUSSION

The increased TC and LDL-C, and reduced TG observed in rabbits fed 10% egg yolk + 2% garlic, 20% egg yolk + 2% garlic and 20% egg yolk + 4% garlic powder (figures 6, 7 and 8) is similar to the investigation reported by [20]. Although, the present study diet composition did not include olive or corn oil as contained in the AIN-76 diet formulation of [20]; higher dose of garlic powder was used (4%). However, similarities exists between the results of some selected lipid profile (TCs, LDL-Cs and TGs) obtained in the present study and the previous report of [20], despite the difference in diet composition, quantity of garlic dose and sample used. The report of [20] on serum HDL-C did not agree with the present study. The report of [13] and [18] is however in line with the result of whole blood HDL-C in the present study. Reference [13] have reported increased plasma HDL-C of subjects fed 3 or 6 egg per day in addition to their habitual diets for 3 months. Reference [18] reported that day old chicks fed graded levels of garlic powder (1, 3 and 5%) for 35 days resulted in HDL-C ($P<0.05$) increased. Reference [21] also reported increased TC, HDL-C, LDL-C and reduced TG in 75 normo-lipidaemic volunteers (adults) assigned 10.8gm alliin tablets per day in 12 weeks. Although, its effect was not significant and as such, garlic was regarded as clinically irrelevant in lowering serum lipid. In contrast, [14] reported reduced serum TC, HDL and LDL-Cs in forty hyperlipidemic adults that consumed three medium hardboiled eggs daily

for 6 weeks. The report of [12] on some selected lipid parameters (TC, HDL-C, LDL-C and TG) in serum concentration of individuals that consumed one egg per day for 5 weeks is completely different from the finding in the present study. [12] reported that consumption of one egg per day had no effect on the considered lipid parameters. The addition of graded levels of garlic powder in egg yolk supplemental diets fed to rabbits did not display any hypocholesterolemic effect in the present study contrary to the report of [18]. The report of [15] on plasma TC and TG of quails fed 1, 2 or 4% garlic powder supplementation for 12 weeks is also contrary to the present study. The reason for the contrasting report could be due to the quantity of garlic and egg yolk supplementation, choice of sample for the assessment of lipid profile, experimental subjects used and perhaps preparation of garlic and egg yolk. The reduced TGs observed in rabbits fed graded levels of egg yolk mixed with garlic powder is suggested to have resulted from increased TCs. This perhaps repressed the synthesis of LDL-receptors (endocytotic uptake of LDL particle) that releases fatty acids into the cytosol from the lysosomal degradation of cholesteryl esters; the fatty acids are made available to glycerol-3-phosphate for the synthesis of triacylglycerol (TG) in line with the report [22].

CONCLUSION

In the current study, graded levels of egg yolk supplementation (10 or 20%) did not significantly affect the whole blood cholesterol. In other word, supplementation of growers mash with graded levels of egg yolk did not significantly induce hypercholesterolemia. However, increased animal body weight was observed in rabbits fed 10 or 20% egg yolk supplementation. Graded levels of garlic powder fed to rabbits did not demonstrate cholesterol lowering property. However, the finding in the present study is contrary to the general belief that garlic possesses strong hypocholesterolemic properties. Increased levels of garlic powder supplementation resulted to increased (whole blood) concentration of LDL-Cholesterol (speculative atherosclerotic condition). Furthermore, the increased supplementation of a combination of graded levels of egg yolk and garlic powder led to increase in whole blood cholesterol which indicates that addition of garlic powder at levels used in this study did not show cholesterol-lowering property. In view of the results obtained from the present study, dietary hen egg yolk and garlic powder supplementation did not significantly affect blood lipid profile. Hypothetically, that is to say consumption of 15-30 grams, equivalent to 3-4 hen egg yolk per day will not result to hypercholesterolemia.

Similarly, [23] argues that the cholesterol in the yolk is not what causes a problem, because fat (in particular, saturated) is much more likely to raise cholesterol levels than the actual consumption of cholesterol. In further studies, it is suggested that larger quantity of egg yolk can be used to induce hypercholesterolemia and supplementation of large dose of freshly prepared garlic extract could perhaps lower blood egg yolk-induced hypercholesterolemia. Dose response and toxicity of garlic when taken in large quantity could also be taken as a subject for further investigation.

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