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Lipoprotein lipase gene expression profile of broiler chicken breeds administered varying levels of ginger extract in drinking water

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ABSTRACT: This study investigated the Lipoprotein Lipase gene expression profile of broiler chicken breeds administered varying levels of ginger extracts in drinking water. A total of 270 day-old unsexed broiler chicks comprising Arbor acre Plus, Cobb 500 and Ross 308 breeds were randomly allotted into three treatments of 90 birds each, with each of the treatments comprising three blocks of 30 birds of each of the three breeds, replicated into three with 10 birds per replicate in a randomised complete block design arrangement. The Birds in T1 (control had 0.2 g of Oxytetracycline in 2 litres of water). A 200 ml solution of 8 ml of undiluted ginger extract and 192 ml of water was constituted as T2 (4 %). Another 200mls solution of 12mls of undiluted ginger extract added to 188mls of water was constituted as T3 (6 %). The birds were fed a single-phase diet of 22.34% CP and 2948.05 Kcal/KgME for 8 weeks. After slaughter, the guanidinium thiocyanate-phenol-chloroform method was used to extract genomic RNA from the abdominal fat tissue samples. The extracted RNA was converted to cDNA using the FIREScript RT cDNA Synthesis KIT. The synthesised cDNA was amplified using the My IQ single colour real-time thermo-cycler to ascertain the cyclic threshold values used in computing the fold change values. The results showed significant ($p<0.05$) differences in the Lipoprotein Lipase gene expression values obtained for the different breeds of broiler chickens administered 0 %, 4 % and 6 % ginger extract. The Lpl gene in Cobb5 was highly up-regulated with a fold change value of 2.48, while it was rather downwardly regulated in Abor acre plus and Ross 308 strains of broiler chickens with fold change values of -1.86 and -0.55, respectively, when administered 0% aqueous ginger extract. However, the Lpl gene was up-regulated in the three strains of broiler chicken administered 4% aqueous ginger extract. Ross 308 was highly upregulated with a fold change value of 4.66, followed by Arbor acre plus with the fold change value of 0.69, while Cobb 500 was the least with the fold change value of 0.1. More so, the Lpl gene in Arbor acre plus and Cobb500 were up-regulated with the fold change values of 1.18 and 2.09, respectively, while Ross 308 had a downward regulation with a fold change value of -1.29 when administered 6% aqueous ginger extract. The study showed that administering aqueous ginger extract regulated the expression of Lipoprotein Lipase gene differently in the different types of broiler breeds examined, indicative of ginger extract fat fat-modulating ability necessary for the production of lean and less fatty meat.

Keywords: Breeds, gene expression, ginger extract, lipoprotein lipase, varying levels.

INTRODUCTION

The quest to attain quick market weight by poultry farmers has resulted in the feeding of broiler chickens with formulations that left resultant huge fat deposits in the broiler chicken meat. This quest could as well make farmers place preference on certain breeds of broiler chickens over others, based on their perceived performance and meat quality characterisation. Over the years, consumers have shown a continued preference for

lean broiler chicken meat over fatty broiler chicken meat (Gugu *et al*., 2022). This preference has also been reported to be due to a combination of health, nutritional, organoleptic, processing and storage factors (Petracci *et al.*, 2015). The authors further stated that lean broiler meat, with a higher protein-to-fat ratio, is vital for muscle growth, metabolism, and overall health of the consumers. Das and Ingole (2023) reported that excess intake of fatty meat increases low-density lipoprotein levels, which play a critical role contributing to most coronary heart diseases. Huang and Ahn (2019) reported that lipid oxidation is a primary factor in meat spoilage, and lean meat having less fat shows slower deterioration. The authors further stated that fatty meat tends to oxidise more rapidly during storage, leading to rancidity and off-flavours, thus reducing the shelf-life and consumer acceptability. Li and Liu (2012) reported that the presence of exogenous antioxidants in the animal diet can increase the stability of the lipid in meat.

One of the natural antioxidants which has been found to enhance performance characteristics in poultry is ginger (Dieumou *et al.,* 2009). Ademola *et al*. (2004) stated that ginger possesses a mixture of three major chemical components: gingerols, zingerone and shogaols, which may be responsible for its different biological activities, such as antibacterial, antidiarrheal, antifungal, antioxidant, and as a growth promoter. The preference for lean meat by most consumers over fatty meat has also encouraged the use of organic substances such as ginger in broiler production. Barazesh *et al.* (2013) reported a decrease in abdominal fat in broiler chickens fed with diets containing ginger. Research conducted *in vitro* shows that ginger extract might control the quantity of free radicals and the peroxidation of lipids and have anti-diabetic properties (Morakinyo *et al.,* 2011). Eltazi (2014) reported that the control group had the highest percentage of abdominal fat, and that the inclusion of ginger in the diet was inversely related to fat levels.

The lipoprotein lipase (LPL) gene is one of the lipid-regulating genes that has been reported to play a critical role in controlling triacylglycerol partitioning between adipose tissue and muscles that increases fat storage and provides energy in the form of fatty acids for muscular growth and enhancement of meat sensory attributes (Otu *et al*., 2024). In broiler chickens, heightened LPL gene activity has been reported to be associated with significant peripheral fat deposition, highlighting its impact on lipid metabolism and storage (D'Andre *et al*., 2013). While ginger clearly influences fat metabolism and antioxidant systems, there is a paucity of information on the molecular insights of the expression pattern of this gene, which is thought to be modulated by the concentration of ginger extract, served to different breeds of broiler chickens in drinking water. This study was therefore designed to determine the lipoprotein lipase gene expression pattern of broiler chicken breeds administered varying levels of ginger extract in drinking water.

MATERIALS AND METHODS

Experimental location

This research was carried out at the Poultry Research Unit of the Department of Animal Production, School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State, Nigeria. Minna is the capital city of Niger State, which lies between latitude 9°37'N and 6°37'N, longitude 3°30' and 7°20'E. Its average annual rainfall is 1229 mm and an average temperature which ranges from 23-34°C with relative humidity of about 77 % (Njoku *et al.* 2021). The state's entire landscape is covered by the Southern Guinea Savannah vegetation. The laboratory analysis was carried out at the Department of Animal Production Laboratory, Federal University of Technology, Minna, while the molecular analysis were carried out in African Biosciences Laboratory, JaaGee House, Ibadan-Ife expressway, Ibadan, Oyo State, Nigeria.

Experimental materials and their sources

Three commercial strains of day-old unsexed broiler chicks were used for the experiment. Fresh ginger rhizome, commercial antibiotics, formulated diet and laboratory reagents were also used in this research. The feed ingredients for the formulation of the broiler diets (Maize, Soybean cake, Fish meal, Wheat offal, Palm oil, Limestone powder, Bone meal, Salt, Lysine, Methionine, Premix and Toxin Binder), medication (Oxytetracycline®) were purchased from reputable distributors in Minna. The Ginger rhizome was purchased at Kure ultra-modern market, located along the Old Airport Road, Minna. The birds were purchased from an accredited dealer within Niger State, Nigeria.

Experimental design and diet

A total of 270-day-old broiler chickens' strains (Arbor Acre Plus, Cobb 500 and Ross 308) were used for this experiment. The birds were randomly allotted into three treatments of 90 birds each, with each of the treatments comprising three blocks of 30 birds of each of the three strains, replicated into three with 10 birds per replicate in a randomised complete block design arrangement. Birds in T1 (control had 0.2 g of Oxytetracycline in 2 litres). A 200 ml solution of 8 ml of undiluted ginger extract and 192 ml of water was constituted as T2 (4 %). Another 200 ml solution of 12 ml of undiluted ginger extract was added to 188 ml of water, was constituted T3 (6 %). The diets used for the experiment were formulated and compounded in accordance with the recommendations of the National Research Council (NRC) (1994). The diet had a metabolizable energy of 2,800 kcalME/kg and a crude

protein content of 22 %. Feed and water were served to the birds *ad libitum* throughout the eight-week duration of the study.

Preparation of ginger extract

The fresh ginger rhizome was washed with clean water to remove dirt and debris, peeled and sliced into pieces and ground into mash using a blender (Polystar electric blender, Model PV-BL999B). The mash was put into an extractor to squeeze out the concentrated ginger juice. The drained ginger juice was stored in a bottle and kept in a refrigerator until needed, as recommended by Paudel (2022).

Management of the experimental birds

The birds were housed in deep litter pens during the period of the study. Prior to their arrival, the pens were washed and disinfected against parasites using Vinkokill at 150 ml per 20 litres of water. Litter material that is (wood shavings) was evenly spread on the floor to a height of 30 cm. Drinkers and feeders were also washed and disinfected with Vinkokill. Upon arrival of the chicks, they were weighed individually to obtain their initial weights and after which they were randomly distributed to the various treatment groups. They were provided with anti-stress (vitalyte) through the drinking water. Each tablespoon (20 g) was diluted into 10 litres of water and then administered. The pens were kept at about 35°C during the brooding period and were gradually reduced to about 21°C. Lasota vaccine was administered twice at the 7th and 21st days, while Gumboro vaccine was given at the 14th and 28th days. Five hundred doses of these vaccines were diluted in five litres of water in which a sachet of powdered Peak milk had been added in order to neutralise the presence of any trace of chlorine and administered after the birds had been starved of water for 12 hours (7 pm to 7 am). Feed and clean water were provided *ad libitum* all through the eight (8) weeks of the experiment. Other routine operations, which included washing of drinkers, cleaning of feeders and observation of the birds, were also carried out.

Tissue sample collection and handling

One bird from each of the replicates, in each of the blocks of each of the treatments, making a total of 27 birds, was randomly picked for tissue sample collection. A total of 27 tissue samples were collected from the abdominal fat of the birds into plain bottles and immersed in RNA-Later (an aqueous non-toxic tissue and cell storage reagent that stabilises and protects cellular RNA intact). The samples were properly labelled and transported to African Biosciences Laboratory, JaaGee House, Ibadan-Ife expressway, within 24 hours of collection in an ice-block cooled box, where they were kept in a deep freezer.

Data collection

The following data were collected in the course of this study

Extraction and purification of lipoprotein lipase gene/cDNA

The 27 abdominal tissue samples from the broiler chickens collected were completely submerged in RNA-Later solution, stored in a plain bottle and kept refrigerated until required for use. The guanidinium thiocyanate-phenol-chloroform method was used to extract genomic Ribonucleic acid (RNA) from the abdominal fat tissues as described by Egena *et al*. (2023) and Otu *et al*. (2024) using the following forward and reverse primers shown in Table 1.

Lipoprotein lipase expression/cycle threshold values determination

The extracted RNA was converted to complementary Deoxyribonucleic acid (cDNA) using the FIREScript RT cDNA Synthesis KIT. The process involved using 1µl of Reverse Transcriptase, 2 µl of 10x reaction buffer, 0.5 µl RNase Inhibitor (Ribogrip), 0.5µl of primers with a 5-µM concentration and 10 µl of the RNA sample(at 50ng/µl). Nuclease Free Water was used to balance the reaction volume to 20µl. The thermocycling conditions were as follows: annealing at 25°C for 10minutes, Reverse Transcription at 45°C for 30 minutes and enzyme inactivation at 85°C for 5 minutes. The synthesized cDNA was amplified using the My IQ single colour real-time cycler. The quantitative polymerase chain reaction mixture (qPCRmix) used was Solis Biodyne 5x HOT FirePol qPCR supermix plus. The reaction was done in 25 µl reactions consisting of 4 µl of the 5x HOTFirepolqPCR Mix, 0.4 µl each of the forward and reverse primers and a specific probe which had a concentration of 250nM, 18.2 µl of Nuclease free water and 2 µl cDNA template(100ng). The cycling conditions were as follows: initial activation at 95°C for 12 minutes, denaturation at 95°C for 15 seconds, annealing at 55 and 53°C for 20 seconds (for lipoprotein lipase and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), respectively) and elongation at 72°C for 20 seconds.

Data analysis

Data generated during the course of the study were

Table 1. Lipoprotein lipase gene primer design and description.

Gene name	Accession number	Primer sequence	Primer length	Product length	Exon-exon junction
Lipoprotein lipase (LPL), mRNA	NM_205282.2	Forward – GCGACTCAGTTCTACTTCGTG Reverse – TTCATCTCAGCTTCGGGATCG	21	250	Yes

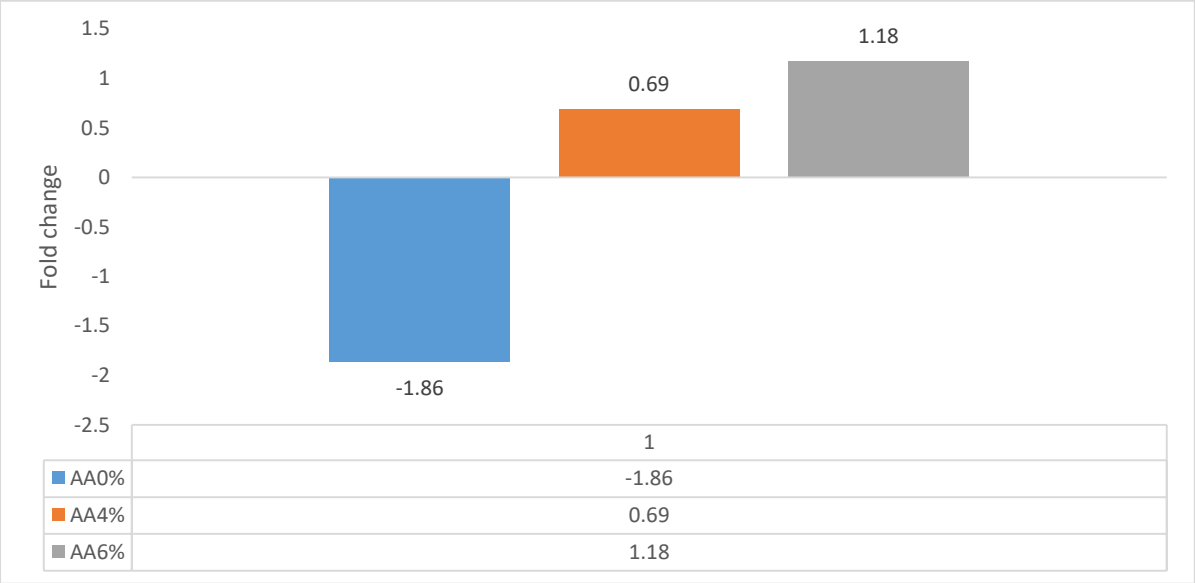

Figure 1. Expression of lipoprotein lipase (Lpl) gene in Arbor Acre plus administered varying levels of ginger extract in drinking water.

subjected to a two-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS, 2007) software. Where significant differences were observed between the means, Duncan Multiple Range Test was used to separate them, at a probability level of $p<0.05$.

RESULTS

Effect of administering varying levels of aqueous ginger extract on the lipoprotein lipase (Lpl) gene expression of broiler chicken strains

The results of the effect of administering varying levels of aqueous ginger extract on the lipoprotein lipase (Lpl) gene expression of broiler chicken strains are presented in Figures 1, 2 and 3. The results in Figure 1 showed the expression of Lpl gene in Arbor Acre Plus broiler chickens. The results showed that there were significant ($p<0.05$) differences in the expression of the Lpl gene administered varying levels of aqueous ginger extract. The Lpl gene was upregulated in the broiler chickens administered 6 % aqueous ginger extract (1.18 fold change), followed by those administered 4 % (0.69 fold change). However, there was a downward regulation of Lpl gene observed in Arbor Acre Plus birds administered 0 % aqueous ginger extract (-1.86fold change).

The results in Figure 2 show the expression of Lpl gene in Ross 308 strain of broiler chickens administered varying levels of aqueous extract of ginger. The results of the expression pattern showed that there were significant ($p<0.05$) differences in Ross 308 strains of broiler chickens. There was upregulation of the Lpl gene in broiler chickens administered 4% aqueous ginger extract (4.66 fold change), while the groups administered 6 % (-1.29 fold change), and those administered 0 % (-0.55 fold change) recorded downward regulation.

Figure 3 showed the results of the expression of Lpl gene in the Cobb 500 strain of broiler chickens administered varying levels of aqueous ginger extract. The results showed significant ($p<0.05$) differences in the expression values obtained for the Cobb 500 strain of broiler birds; the Lpl gene was upregulated in the Cobb 500 strain administered all the varying levels of ginger extract. However, the regulation was higher in broiler chickens administered 0 % aqueous ginger extract in drinking water (2.48 fold change), followed by those administered 6%

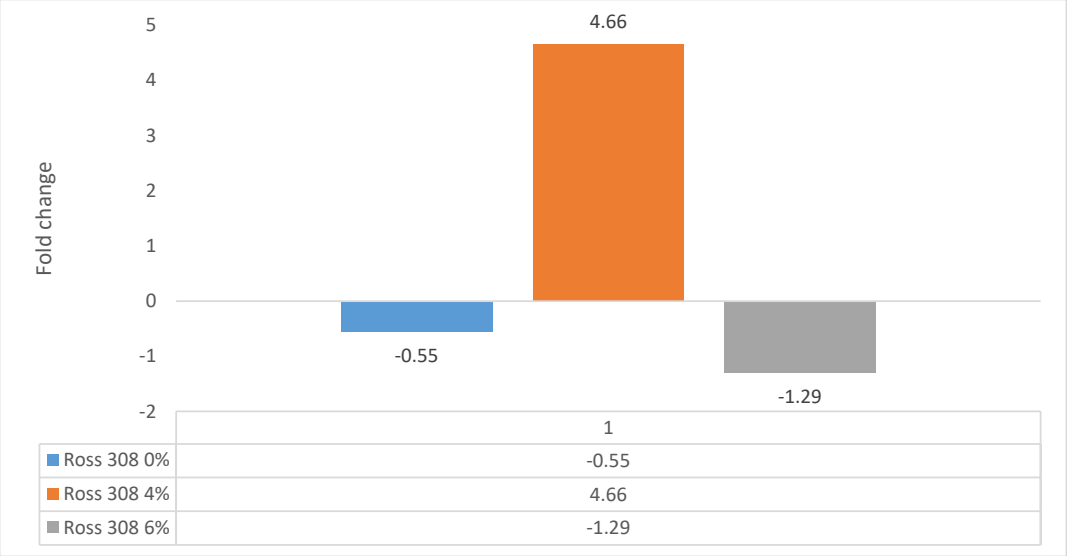

Figure 2. Expression of lipoprotein lipase (Lpl) gene in Ross 308 administered varying levels of ginger extract in drinking water.

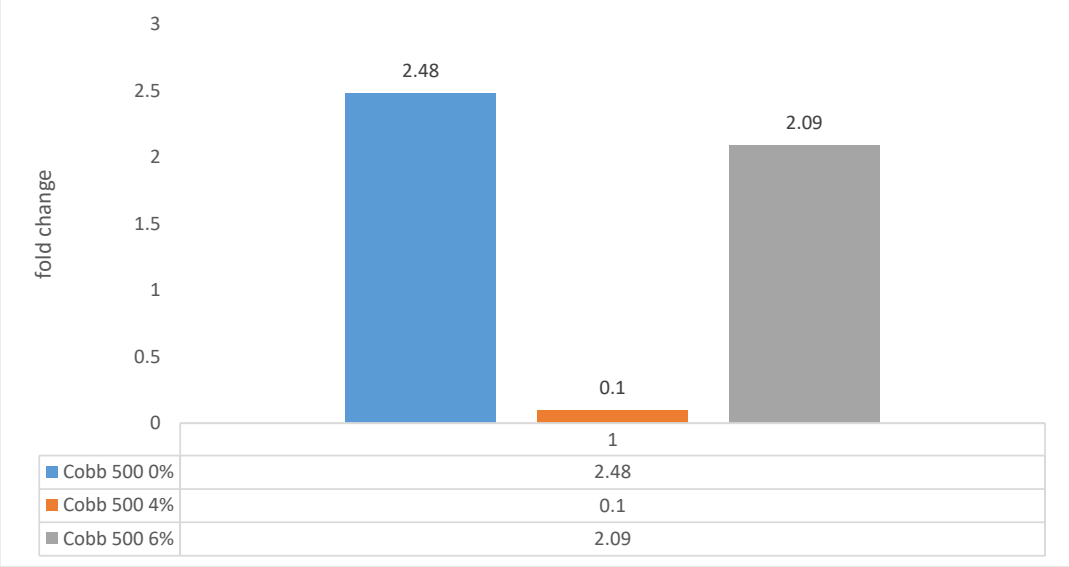

Figure 3. Expression of lipoprotein lipase (Lpl) gene in Cobb 500 administered varying levels of ginger extract in drinking water.

aqueous ginger extract in drinking water (2.09 fold change) and lowest in birds administered 4 % of the aqueous extract (0.10 fold change).

Effect of strains on the expression pattern of Lipoprotein lipase (Lpl) gene of broiler chickens administered different levels of ginger extract in drinking water

The results of the effect of strains on the expression pattern of Lipoprotein lipase (Lpl) gene of broiler chickens administered different levels of ginger extract in drinking water are shown in Figures 4, 5 and 6. The results showed significant ($p<0.05$) differences in the expression values obtained for the different strains of broiler chickens administered 0%, 4% and 6% ginger extract used for the experiment. In Figure 4, the Lpl gene in Cobb5 was highly up-regulated with a fold change value of 2.48, while it was rather downwardly regulated in Abor acre plus and Ross 308 strains of broiler chickens with fold change values of -1.86 and -0.55, respectively, when administered 0%

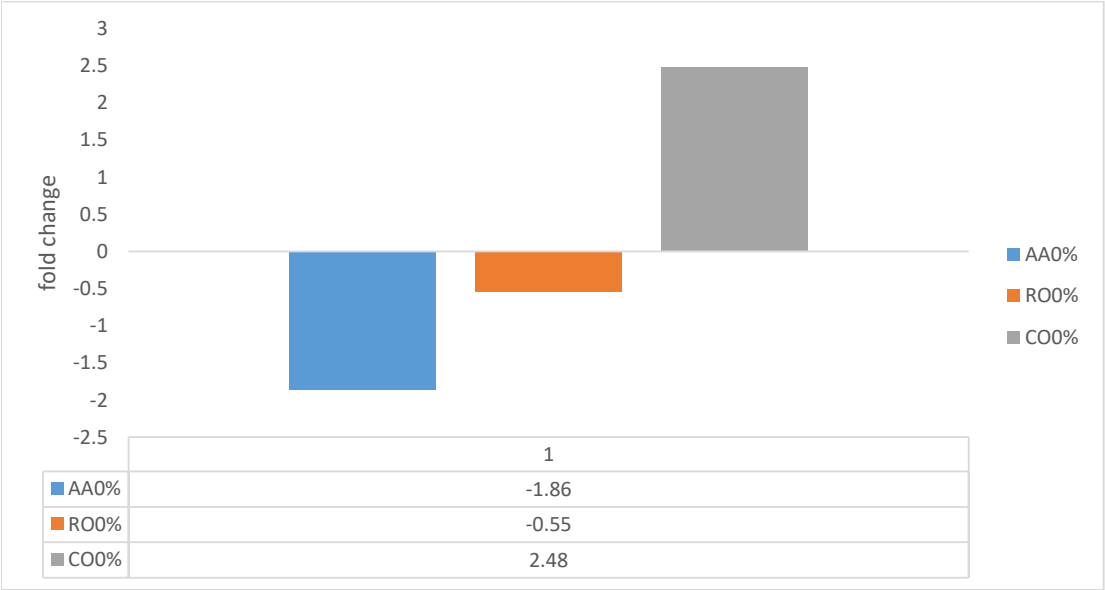

Figure 4. Expression of Lpl gene in broiler chicken strains administered 0% level of aqueous extract of ginger.

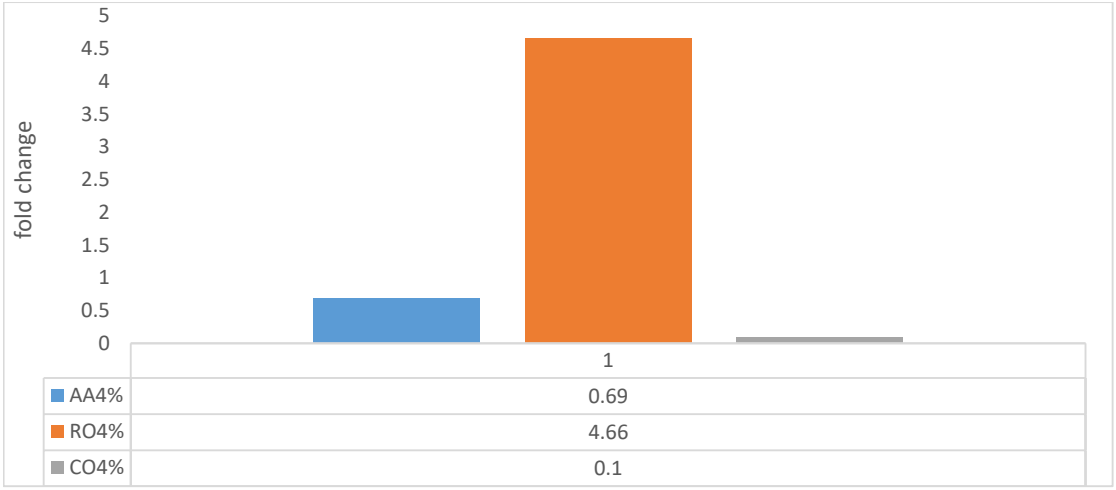

Figure 5. Expression of Lpl gene in broiler chicken strains administered 4% level of aqueous extract of ginger.

aqueous ginger extract. The result in Figure 5 showed that the Lpl gene was up-regulated in the three strains of broiler chicken administered 4% aqueous ginger extract. However, Ross 308 was highly upregulated with a fold change value of 4.66, followed by Arbor acre plus with the fold change value of 0.69, while Cobb 500 was the least with the fold change value of 0.1. Figure 6 showed that the Lpl gene in Arbor acre plus and Cobb500 were up-regulated with the fold change values of 1.18 and 2.09, respectively, while Ross 308 had a downward regulation with a fold change value of -1.29 when administered 6% aqueous ginger extract.

DISCUSSION

Effect of administering varying levels of aqueous ginger extract on the Lipoprotein lipase (Lpl) gene Expression of broiler chicken strains

The Lpl is an enzyme responsible for the hydrolysis of core triglycerides in chylomicrons and very low-density lipoproteins, thereby regulating the supply of fatty acids to the various parts of the body/tissues for either storage or oxidation. Studies have shown that chicken LPL is a crucial enzyme in the regulation of fat accumulation in the adipose

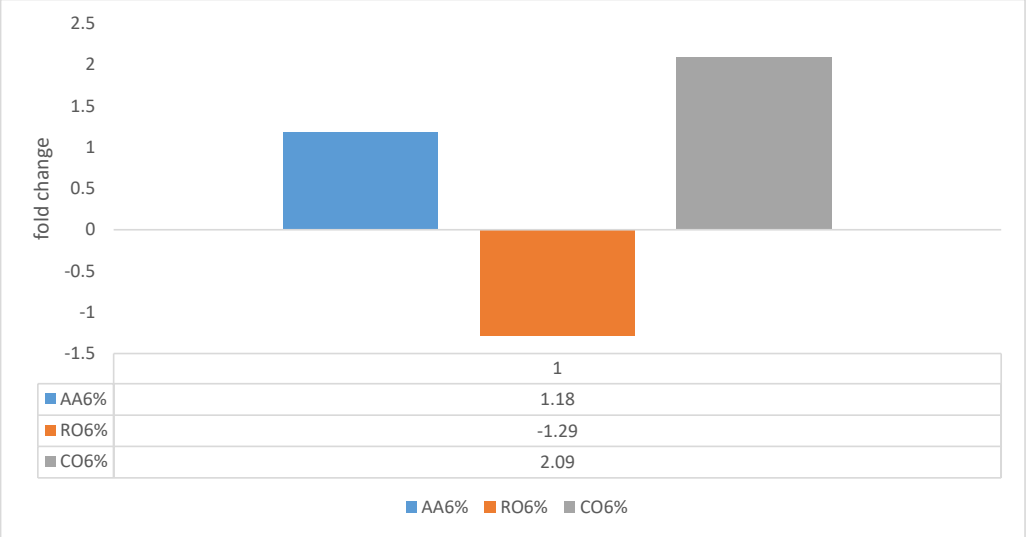

Figure 6. Expression of Lpl gene in broiler chicken strains administered 6% level of aqueous extract of ginger.

tissues of chickens (Sato *et al*., 1999). The LPL gene was much more upregulated in birds administered 4% and 6 % aqueous ginger extract compared to those administered the control. This upward regulation could have been due to the high activities of the Lpl gene in the broiler birds administered these doses of ginger extract. This result is in line with the findings of Naidu *et al*. (2016) that administration of gingerol resulted in a significant reduction in body weight gain, which could be a result of activated adenosine monophosphate protein kinase and adipose programmes that promote triglyceride clearance, not necessarily increased fat storage in adipose tissues. The downward regulation of the LPL gene in the different strains of broiler chickens administered 0 % aqueous ginger extract could be a result of the reduced activity of the gene as a result of reduced amount of abdominal fat. The findings of this study could be reflective of the depressed feed intake of the broilers in the absence of the ginger extract, which could not predispose them to fat deposition, a necessary precursor for lpl gene fat modulatory activities. This agrees with the findings of Agarwal (1996) and Sharma *et al*. (1996), who reported that medicinal herbs such as garlic and ginger possess lipid-lowering effects, and their absence makes the tissue environment more likely to suppress lpl gene activities. Therefore, it can be deduced that the administration of ginger extract regulated the expression of the LPL gene of broiler chickens, thereby giving rise to the production of lean, less fatty chicken meat.

Effect of strains on the expression pattern of Lipoprotein lipase (Lpl) gene of broiler chickens administered different levels of aqueous ginger extract in drinking water

The up-regulation of the Lpl gene was in the order of Ross 308 > Arbor acre plus > Cobb 500. The differences observed in the expression of the gene may be due to the genetic constitution of the different breeds, given that different breeds of broiler have unique growth patterns and, by extension, intermuscular and abdominal fat concentration, which are triggers to the activities of Lpl gene. This result is in line with the findings of Faulconnier *et al*. (2001) and Bonnet *et al*. (2000), who purported that LPL expression may be regulated by different mechanisms in different animal species. Also, Ren *et al*. (2002) reported that higher LPL gene expression in abdominal fat depots was correlated with higher fat content in dairy cattle. With the findings above, it can be suggested that Lpl expression in broiler chicken strains is correlated with the rate of fat accumulation.

Conclusion

From the findings of this work, it can be concluded that administration of aqueous ginger extract up to 6% in the drinking water for the three different strains of broiler chickens significantly expressed the gene. However, Ross 308 strains of broiler chickens should be administered 4% aqueous ginger extract in drinking water for better expression of Lpl gene, while 6% should be administered to Arbor acre plus and Cobb 500 broiler chickens as this will enhance the exploitation of lipid lowering agents in ginger in reducing abdominal, muscle and thigh fat of broiler chickens, since fat accumulation predisposes individuals to cholesterol.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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