ISSN 1682-8356
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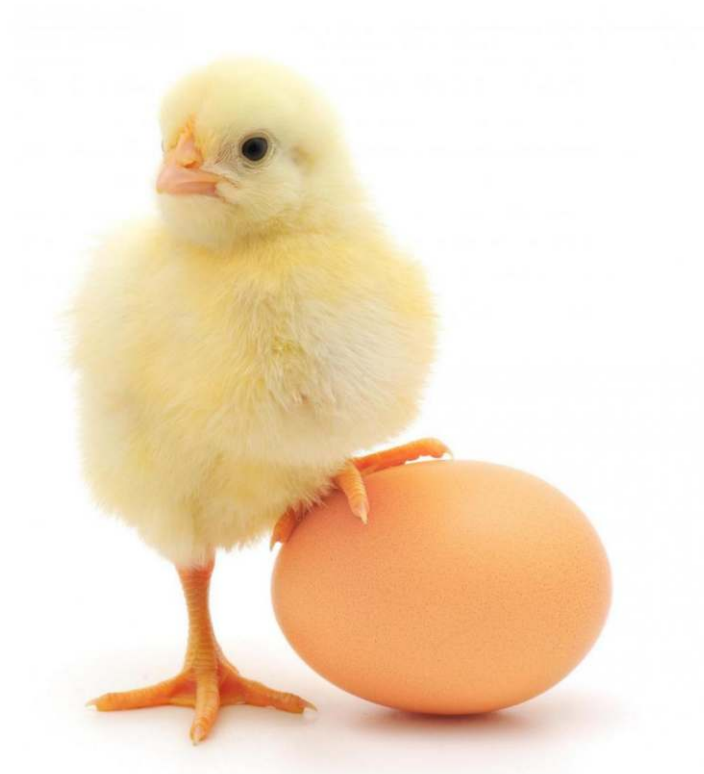

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 10 (7): 567-573, 2011
ISSN 1682-8356

Lipid Profile of Broilers Fed Zinc Bacitracin on Plant and Animal Protein Diets

Benson Ogboko
Department of Biomedical Sciences, University of the Western Cape,
Private Bag, X17 Bellville, 7535 South Africa

Abstract: This investigation was undertaken to determine the effect of protein sources and growth promoter Zinc bacitracin on the lipid profile of broiler chickens. Two trials were conducted, comprising of 240 day-old chicks and 120 day-old chicks respectively, they were housed in battery cages in a broiler pen, randomly distributed into four (4) major groups each receiving diets with protein derived from plant or animal protein with or without the addition of Zinc bacitracin. The plant and animal diets were compiled, milled and analysed, before been subdivided into two subgroups with Zinc bacitracin included at 300 g per tonne. The feeding trial lasted for six (6) weeks. On terminating the feeding trial, 30 marked birds from each group were slaughtered and blood samples were analysed for, total cholesterol, triglyceride, HDL and LDL. There was a significant difference ($p<0.05$) in the broiler plasma cholesterol levels, as observed in total cholesterol, HDL and LDL cholesterol levels while there was no significant difference in the triglyceride levels. Animal protein diet induced the highest cholesterol levels but on addition of Zinc bacitracin the level was reduced, as was also the case in plant diets. Reduction of plasma cholesterol levels on the inclusion of Zinc bacitracin could indicate that cholesterol is been deposited in the tissues.

Key words: Zinc bacitracin, feed, broilers, cholesterol

INTRODUCTION

Fatty acids or glycerol esters differ from carbohydrates in that they have low oxygen content (Malden *et al*., 1979; Lopez *et al*., 2011; Das, 2008). They contribute up to 17% of dry body weight of mature broilers, they serve as a source of energy for animals and are often associated with the following compounds; steroids, waxes and phospholipids. Fatty acids are classified as essential and non-essential fatty acids (Das, 2008).

Those that can be synthesized by the animals are the non-essential fatty acids and those that cannot be synthesized by the animal tissue and must be present in the diet are termed the essential fatty acids. In poultry linoleic acid must be present in the diet of young growing chicks or they will grow poorly, they will generally show an accumulation of liver fat and be more susceptible to respiratory infections, such as new-castle disease, mycoplasmosis and infectious bronchitis (Malden *et al*., 1979; Daghir *et al*., 2003)

Fats in the diet provide fatty acids necessary for the structure of cell membranes and prostaglandin's. They also acts as solvents for many substances including vitamins A, D, E and K. High food fat content can, however be detrimental to consumers as it can lead to numerous degenerative diseases (Veith, 1993; Das, 2006). In this regard, saturated fatty acids and unsaturated fatty acids, have been shown to exert different effects, with the unsaturated fats being more unstable and prone to oxidation and thus contributing to the production of free radicals, while saturated fats are in turn linked to cardiovascular diseases (Das, 2006).

Saturated Fatty Acids (SFAs) consist of hydrocarbon backbones, which terminate in a carboxyl group that cannot be hydrolysed (-COOH), they contain no double bonds and in animal tissues, the most abundant varieties have 12, 14, 16 and 18 carbons (Lauric acid, myristic acid, palmitic acid and stearic acid respectively). Unsaturated fatty acid are those having double bonds between carbon atoms and those containing more than one double bond (poly-unsaturated fatty acids), such as linoleic and linolenic acid, there are commonly found in plants (Veith, 1998; Satya *et al*., 1990; Warensjo *et al*., 2008).

In the last decade, there has been increased evidence of the negative influence of high fat diets on hypertension, cardiovascular diseases, type II diabetics and some forms of cancer (Malden *et al*., 1979; Das, 2008). With up to 17% of dry body mass of broilers being made up of fat, high consumption ratio of this food in modern diets can contribute to particularly cardiovascular diseases in consumers. Modern diets are profoundly different from what was consumed a few decades ago. Meeting the demands for protein from animal sources also increases the fat consumption. There is a linear relationship between cardiovascular diseases, mortality and serum cholesterol and total saturated fat levels in the diet. In view of this, there have been calls to make more in depth studies on the relationship between diet and health (Nevin and Scrimshaw, 1996; Du *et al*., 2010).

Poultry also contributes to cholesterol intake and as plasma cholesterol concentration is controlled by several factors, including the entry of dietary cholesterol into the circulatory system and the clearance of lipoprotein from the circulation through receptor dependent and independent pathways (Jackson *et al*., 1994). It is necessary to ascertain how various poultry feeds can affect cholesterol levels in the broilers.

Fatty acids are transported in the plasma mainly as esters in Phospholipids (PL), Cholesterol Esters (CE) and triglycerides associated with plasma lipoproteins. Dietary fatty acids undergo elongation, de-saturation of fatty acids and other reactions to produce a further spectrum of acids (Horrobin, 1995). The distribution of fatty acid among the different lipoprotein fractions depends to a large extent on metabolic events, such as exchange and transfer reactions (Fieding, 1993) and it also depends on the composition of the dietary lipid (Rideout *et al*., 2010).

The uptake of lipid by the peripheral tissues occurs predominantly through Low-Density Lipoprotein (LDL), particularly in its partially oxidized form, by the action of scavenger receptors (Krieger, 1997). While many studies have examined the fatty acid composition of plasma in relation to Coronary Heart Disease (CHD), little information is available on the fatty acid composition in LDL.

Cholesterol levels in the body are largely controlled by the liver, whether it be cholesterol in the bloodstream or in the organs and since cholesterol is insoluble in water (therefore insoluble in plasma), it is carried within a lipid wrapper that has water soluble proteins on its surface, thus enabling the lipid/cholesterol complex to move through the blood (Jacque *et al*., 1999). These lipid-complexes may be either LDL (low density lipoproteins) or HDL (high density lipoproteins). HDL, is found in the plasma and lymph and is predominantly involved in reverse cholesterol transport and mediates the removal of cellular cholesterol by the passive re-absorption of membrane cholesterol from the plasma membrane. It is known as “good cholesterol” it tends to function in moving excess cholesterol to the liver for breakdown. Conversely LDL carries dietary cholesterol to the liver and other organs and causes cholesterol deposition. LDL acts as a preferential oxidative substrate over HDL particles and might protect HDL particles from oxidation. HDL also absorbs lipo-polysaccharide toxins and may prevent the vascular collapse seen in Endo-toxin shocks (Jacque *et al*., 1999; Hoekstra *et al*., 2010).

In a situation of too much LDL (more than the liver and organs can use), they are deposited on the cells of the artery walls, potentially leading to arteriosclerosis, also LDL is associated with vascular disease, as these molecules tend to filter through the arterial walls whereas HDL attracts cholesterol out of the wall and transports it to the liver where it is metabolised. Consumption of high animal fat can lead to increased levels of the LDL cholesterol fraction (Veith, 1998; Ulbrich and Southgate, 1991). There is a consensus among nearly all investigation and expert groups that the cholesterol carried in LDL is the principle culprit in arterial cholesterol deposition and that the HDL is protective.

Generally, when fat supplies less than 25% of dietary calories, coronary heart disease is rare. Moreover, the dietary content of saturated fat including Trans-fatty acids increases serum cholesterol and causes damage. Contributory factors to cardiovascular diseases are hypertension, obesity and lack of physical activity. Arteriosclerosis does not only lead to heart disease but can also be responsible for strokes and kidney disease (Veith, 1998). Considerable attention has recently been focused on the relationship between dietary fatty acids, the development of Coronary Heart Disease (CHD) and other fatty acids related diseases such as, intra-abdominal distribution of fat in the body (Bouchard *et al*., 1990; Kannel *et al*., 1991; Matsuzawa *et al*., 1995; Warensjo *et al*., 2008; Rideout *et al*., 2010).

Diets rich in grains, vegetables and fruits are considered to be protective there is speculation and some evidence that dietary fibre, dietary antioxidants such as vitamins E and C and carotenoids as well as other dietary factors are also involved. Dietary casein has also been shown to produce higher cholesterol levels than soy protein in a number of animal species, including, rats, lambs, guinea pigs, pigs and monkeys (Van and Beynen, 1987; Mirmiran *et al*., 2009). Replacing animal protein with soya bean protein in the diet will effectively lower total cholesterol levels and LDL cholesterol while increasing HDL cholesterol (Vessby *et al*.,1983; Holmes *et al*., 1980; Grundy and Abrams, 1983; Blum *et al*., 2003; Bazzano *et al*., 2001). Also Soya bean protein tends to decrease Triglyceride levels (Verrillo *et al*., 1985).

Dietary protein has various effects on gastrointestinal function for example digestive fluid excretion and gut motility, both of which may affect the absorption rate of nutrients (Hara *et al*., 1992). Secondary bile acids, a product of cholesterol metabolism, have been linked to intestinal tumour formation and plant proteins result in a very low ratio of secondary to primary bile acids. Oats and bean products contain large quantities of water-soluble fibre and are particularly efficient in reducing blood cholesterol levels particularly the LDL cholesterol level, which tend to clog blood vessels (Anderson and Gustafson, 1988; Bazzano *et al*., 2001). By binding cholesterol and bile acids fibre not only reduces cholesterol levels but also protects against colon cancer, as secondary bile acids are carcinogenic (Anderson and Gustafson, 1988). It is known that a variety of animal proteins are hypercholesterolemic.

Rabbits developed hypercholesterolaemia and arthesclerosis when fed cholesterol free diet containing 38% casein (milk protein), as the main protein component and these effects were not observed when the protein was changed to soya protein flour, but addition of casein to the basic diet enhanced the artherogenic effect of added cholesterol. Soya bean flour, however, decreases the incidence and degree of sclerosis in rabbits fed cholesterol (Carroll, 1991; Blum *et al.*, 2003). Fish protein in particular was found to induce severe hypercholesterolaemia when compared to soya bean protein, casein, whey protein and ovalbumin (Beynen *et al.*, 1990).

As poultry are often raised on diets, which include substantial portions of fishmeal, as such poultry could contribute to increased cholesterol consumption by consumers. Soy protein on the other hand reduces triglycerol levels but appears to have little effect on HDL cholesterol levels (Carroll, 1991). An elevated serum triglyceride level is also a risk factor for arteriosclerosis, but this could be, due to high triglyceride levels being associated with low HDL cholesterol levels. When triglyceride metabolism is efficient, triglyceride concentration be lowed while HDL concentration is increased (Albrink, 1991; Jonnalagagadda *et al.*, 1996). Elevated tryglyceride levels will also lead to obesity, which places one in a high-risk category for degenerative diseases. Obesity is characterized by excess body fat accumulation, which is generally associated with enhanced lipid consumption (Fricker *et al.*, 1998). Similar evidence that might be casually linked to obesity comes from cross-sectional analysis indicating that the selection of a diet with a high fat to carbohydrate ratio is associated with obesity (Gazzaniga and Burns, 1991; Bolton-Smith and Woodward, 1994; Verrillo *et al.*, 1985; Irace *et al.*, 2009; Hartwich *et al.*, 2009).

In this study we investigated the role of dietary animal and plant protein sources on lipid profiles in broilers. Since broiler chickens contribute a substantial portion of the human diet, the effect of feed source on lipid composition can have implications for human health. Moreover, since growth promoters often form a component of poultry feed, this study also includes an investigation on the influence of the growth promoter Zinc bacitracin on the lipid profiles of broilers.

MATERIALS AND METHODS

Diets: Plant and animal protein diets were formulated using the standard computerized feed formulation programme (Stelplan Programme, 1999). Used by the Department of Poultry Science, Faculty of Agriculture, University of Stellenbosch, in South Africa. The feeds were compounded at the Mariendahl Animal Research facility by milling one tonne of feed. The milled feed was bagged and labelled. Chicks were used in a repeated feeding trial. The 240 day-old chicks were obtained from a commercial hatchery (Rainbow Chicken Limited, Worcester South Africa); they were then housed (10 chicks per cage) in a single brooder pen, fitted with wire-framed cages, with feeders and automatic drinkers fitted to each cage.

The chicks were grown under controlled temperature (25-27°C) and reduced airflow, with moderate light intensity, provided by (six) 20 watts fluorescent lights. Prior to the bird's arrival, the cages and the entire pen were cleaned, disinfected and fumigated to prevent infections. On arrival the chicks were immediately weighed, sexed and randomly placed into cages at 10 birds per cage. Each group had 60 birds, placed on four different feeding regimes. Each regime was assigned six (6) birds per group of ten replicas in four (4) different regimes (Table 1). Data on feed consumption (Table 2) and growth were pooled. The birds were fed daily at 9.00am and both feed and water were provided *ad-libitum* (Mirosh *et al.*, 1981).

Table 1: Feeding regimes

A	Plant based diet	60 (chicks)	No Zinc bacitracin
B	Animal based diet	60 (chicks)	No Zinc bacitracin
C	Plant based diet	60 (chicks)	With Zinc bacitracin
D	Animal based diet	60 (chicks)	With Zinc bacitracin

Zinc bacitracin (growth promoter) was added to the diet at 300 gm per tonne

The feeding procedure continued for six weeks and on the final day of the feeding trial; the mass gain, total feed consumption and mortality rates during the trial were recorded. The condition of the birds and anatomical abnormalities were also examined. The birds were then transferred to the abattoir for slaughter, organ samples collected and carcass parameters determined. Collection of blood samples and determination of carcass parameters were randomly done on 10 birds. The remaining birds were only used for growth and feed data. For the second feeding trial, the same procedures were followed, except that 120 day-old chicks were used and 30 birds were assigned to each treatment, consisting of three replicas of 10 birds each. At the end of the experiment, 20 marked birds from each dietary regime were selected for analyses. Slaughtering and organ collection was carried out as previously described (Mirosh *et al.*, 1981).

Sample collection and analysis: At the end of the trial, 30 randomly selected birds from each dietary group were used for blood analysis. Blood samples were collected from the jugular vein into heparinised tubes, centrifuged (Spectrafuge centrifuge, 6000 rpm) and the plasma was stored at -15°C. Total cholesterol, HDL-cholesterol, LDL-Cholesterol and triglyceride levels were determined by Chemical pathology routine Diagnostic laboratory at Groote Schuur Hospital situated in Cape Town, South Africa (Baron and Warren, 1997).

Statistical analyses: The Data was subjected to Repeated measures ANOVA, using the Levene's Test of Homogeneity of Variances, of the Statistical Analysis system (SAS, 1982).

Table 2: Composition of plant and anima diet for broiler chicken

Raw materials	**Plant protein diets (kg)**	**Nutrients**	**%**
Lime	1.15	Calcium	0.90%
Cocsidiostart	0.1	Arginine	1.65%
Maize (High grade)	49.159	Isoleucine	1.02%
MonoCalcium	1.225	Leucine	1.96%
Phosphate oil (21 Day)	8.674	Linoleic acid	1.07%
Lycine	0.011	Lycine	1.20%
Methionine	0.2661	Metabolic energy	13.2MJ
Soya cake	39.003	Methionine	0.55%
Salt	0.217	Methionine + Cystine	0.96%
Vitamines/microminerals	0.2	Sodium	0.10%
		Avail. Phosphorus	0.40%
		Avai. Protein	20.92%
		Avail. Threonine	0.80%
		Avial. Tryptophan	0.25%
Raw materials	**Animal protein diets (kg)**	**Nutrients**	**%**
Poultry by-products	5	Arginine	1.25%
Lime	2.452	Calcium	1.90%
Cocsidiostart	0.1	Isoleucine	0.95%
Maize (High grade)	66.617	Leucine	2.34%
Lycine	0.031	Lycine	1.20%
Methionine	0.131	Linoleic acid	1.46%
Soya cake	5	Metabolic energy	13.3MJ
Fish meal	2047.00%	Methionine+Cystine	0.96%
Vitamin/microminerals	0.2	Sodium	0.20%
		Avail. Phosphorus	0.705
		Avail. Protein	25.05%
		Avail. Threonine	0.95%
		Avail.Tryptophan	0.25%

Formulated feed result, (Stelplan Programme, 1999) University of Stellenbosch, Animal Science Department feed formulation techniques

Table 3: Total cholesterol, Low lipoproteins (LDL), High lipoproteins (HDL)

Treatments	Cholesterol (nmol/l)	LDL (mmol/l)	HDL (mmol/l)
A	2.85	0.90	1.90
B	4.13	1.41	2.80
C	2.75	1.00	1.75
D	3.10	1.26	1.77

Mean serum cholesterol levels in nmol/l and mmol/l in treatments (A, B, C and D)

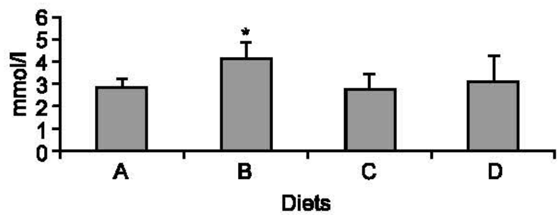

Fig. 1: Total Cholesterol in plasma of broiler chicken feed different dietary regime. Mean plasma total cholesterol levels of broiler chickens fed with different dietary regimes are as follows: plant-based protein source (A), animal-based protein source (B), plant-based protein supplemented with Zinc bacitracin (C) and animal-based protein supplemented with Zinc bacitracin (D) * = ($p<0.01$) significantly different

RESULTS

The total mean serum cholesterol levels in nmol/l and mmol/l in treatments (A, B, C and D) are shown in Table 3. Total plasma cholesterol levels in broiler chickens fed with animal-based protein diet were significantly higher ($p<0.01$) than those fed on the plant-based protein regime (Fig. 1). Treatment B (Animal protein diet without Zn bacitracin) produced the highest total cholesterol level, followed by D, A, C. Regime (B) showed significant ($p<0.01$) reduction in total cholesterol levels when Zn bacitracin was added to the animal-based protein diets (D).

The triglyceride levels were not affected ($p>0.05$). However, broiler chickens receiving animal proteins had higher levels of triglyceride than chicken receiving plant based protein. Plasma HDL was elevated in the broiler chickens fed animal protein (B) ($p<0.05$). This showed mean levels of 2.55 mmol/l, representing slightly higher levels than human standard of (0.7-2.5 mmol/l). Addition of Zn bacitracin to animal protein diet (D) reduced HDL levels significantly ($p<0.05$).

The group fed plant protein (A) showed lower levels with a minimal reduction in HDL levels when Zn bacitracin was added (C). All except one treatment B (animal based diet) levels are within the human standard value for HDL (0.7-2.5 mmol/l). Treatments (A, C and D) showed HDL value (0.7-2.5 mmol/l), which are within the average human standards (Fig. 2). The plasma LDL

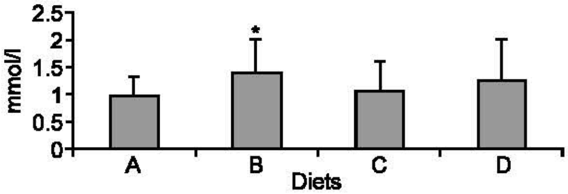

Fig. 2: Mean HDL plasma levels in broiler chicken feed different dietary regime. Mean plasma HDL levels of broiler chickens fed with different dietary regimes are as follows: plant-based protein source (A), animal-based protein source (B), plant-based protein supplemented with Zinc bacitracin (C) and animal-based protein supplemented with Zinc bacitracin (D) * = ($p<0.05$) significantly different

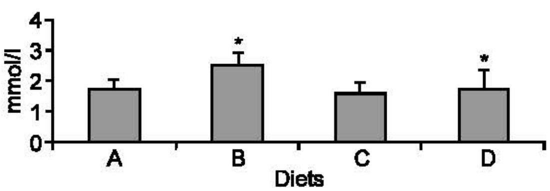

Fig. 3: Mean plasma LDL levels in broilers receiving different dietary regimes. Mean plasma LDL levels of broiler chickens fed with different dietary regimes are as follows: plant-based protein source (A), animal-based protein source (B), plant-based protein supplemented with Zinc bacitracin (C) and animal-based protein supplemented with Zinc bacitracin (D) * = ($p<0.05$) significantly different

levels in broiler chickens fed with animal proteins were significantly higher ($p<0.05$), compared to those fed plant protein diets, which is below the human standard values (1.5-3.5 mmol/l).

Treatment (B) animal protein diet showed the highest value of (1.41 mmol/l), followed by treatment D (1.26 mmol/l), which is when Zn bacitracin is added to the animal protein diet (B) while in plant protein diet (A) the value of (0.90 mmol/l) was increased on addition of Zn bacitracin (C) to (1.00 mmol/l) although non significant. When Zn bacitracin was added to plants protein diet, the levels of LDL was increased (C), but decreases when added to animal protein diet (D) (Fig. 3), similar trend can be noticed in the HDL/LDL ratio, were both plant protein diet and animal protein diets showed a significant ($p<0.05$) lowered HDL/LDL ratio when Zinc bacitracin was added (Fig. 4).

DISCUSSION

The total plasma cholesterol reduction on addition of Zinc bacitracin could imply that Zinc bacitracin leads to cholesterol deposition in the tissues, particularly in

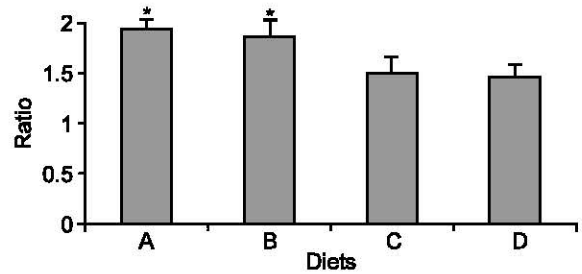

Fig. 4: Plasma HDL/LDL ratio in broilers receiving different dietary regimes. Mean plasma HDL/LDL levels of broiler chickens fed with different dietary regimes are as follows: plant-based protein source (A), animal-based protein source (B), plant-based protein supplemented with Zinc bacitracin (C) and animal-based protein supplemented with Zinc bacitracin (D) * = ($p<0.05$) significantly different

broilers on animal based diets and this could have negative implications on human health (Veith, 1998; Malden *et al*., 1979). One of the most important cholesterol parameters is HDL:LDL ratio as it gives an indication as to whether cholesterol is likely to be deposited in the arteries or not (Jacque *et al*., 1999).

A ratio favouring LDL as noticed when Zn bacitracin is added is considered detrimental to human health and a probable cause of coronary heart diseases, vascular collapse, stroke and kidney disease (Veith, 1998; Jacque *et al*., 1999). Inclusion of Zn bacitracin enhances overall growth, thereby affecting overall metabolism in the broiler chickens. In cholesterol channelling as shown in HDL/LDL ratios, only total cholesterol and triglyceride levels play a role, as they will be absorbed after digestive processes.

The data from this study generally show that broiler chickens fed with plants protein without a growth promoter, produced lower cholesterol levels, and would thus provide the least harmful lipid profiles (Veith, 1998). Low HDL/LDL observed in broiler chickens receiving growth promoter in treatments (C and D), could suggest that cholesterol deposition in the tissues is higher in groups fed Zn bacitracin (C and D), than those without Zn bacitracin (A and B) and may thus be detrimental to consumers. This finding is in harmony with the literature which shows that diets consisting largely of plant proteins will produce the most favourable lipid profiles (Nevin and Scrimshaw, 1996; Jackson *et al*., 1994; Veith, 1993).

Conclusion: The consumption of commercially bred broiler chickens and the possible effect of modern husbandry diets on broilers can portend numerous consequences for the consumer. The fact that 17% of dry body weight of mature broiler chickens is made up of fat and that consumption of it could lead to major health problems, the importance of studying the lipid profiles of broiler chickens, especially when growth promoter (Zn bacitracin) is added to diets can not be undermined. The results suggest that cholesterols are deposited when Zn bacitracin is added to feed. Farmers should be advised not to incorporate Zn bacitracin in their chicken feeds, especially when using animal protein diets. Other researchers have generally supported the finding that dietary animal protein increases the cholesterol levels in the human blood and same can be applicable to chickens particularly with the inclusion of Zn bacitracin in the diets.

Broilers receiving animal protein diet showed higher triglyceride levels in the plasma than broilers receiving plant protein and cholesterol profiles where also significantly higher. This has been implicated in cardiovascular disease in humans.

In verifying the effect of different levels of cholesterol in the broiler chicken blood samples, it was shown that Zinc bacitracin plays an important role in regulating the total cholesterol levels, as well as HDL, LDL and Triglycerol levels. The ratio of HDL/LDL was negatively affected by addition of Zinc bacitracin, particularly in the group receiving animal protein and this could suggest that cholesterol might have been deposited in the tissues.

More investigation on the broiler's tissue cholesterol levels should be carried out as to ascertain tissue level changes upon the inclusion of any form of growth promoter especially Zinc bacitracin to the diets.

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