THE EFFECT OF ZINC BACITRACIN ON THE PHYSIOLOGY OF BROILERS FED PLANT AND ANIMAL PROTEIN DIETS

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Abstract

This study presents the influence of zinc (Zn) bacitracin, a growth promoter on the physiology of broiler chickens fed plants and animal protein diets. The differential effect of the two diets on plasma lipid profiles (total cholesterol, triglycerol, high-density lipoprotein, (HDL) and low-density lipoprotein (LDL), mineral profiles, (including calcium to phosphorus ratio in the broiler chicken bones) and the plasma hormone profile (testosterone, oestrogen and progesterone levels) were investigated. Results showed a significant difference (P<0.05) in the broiler plasma, total cholesterol, HDL, and LDL cholesterol, while there was a non-significant difference in the triglyceride levels. Broilers fed with animal protein diet induced higher levels of cholesterol, but on addition of Zn bacitracin, the cholesterol level was reduced, same was the case with those fed plant protein diets although not significant. Cholesterol levels in plasma were reduced due to addition of Zn bacitracin and could indicate cholesterol deposition in the tissues. Hormonal analysis (oestradiol) showed a significant difference (P<0.05) between plant protein diets and animal protein diets, indicating increase in hormonal plasma with a decrease in oestradiol levels when Zn bacitracin was added especially to plant protein diets. Progesterone levels in plasma were significantly higher in the animal protein diet than in the plant protein diet, but were reduced when Zn bacitracin was added, while Progesterone levels showed a similar decrease (P<0.05) when Zn bacitracin was added in plant protein diet. Testosterone levels in plasma did not increase (P>0.05) when Zn bacitracin was added to the plant protein diet, while there was an increase in testosterone when Zn bacitracin was added to the animal protein diet. Broiler chickens fed with plant protein diet showed significantly higher Calcium levels in bones and claws (P<0.05). The inclusion of Zn bacitracin significantly lowered Ca levels in both diets. Phosphorus levels in bones and claws showed no significant differences. These results indicate that some parameters changed in tissues on inclusion of Zn bacitracin, but its negative effect on the physiology of broilers and the possible effect on humans need to be further investigated.

Key words: Zinc bacitracin, Hormone, Minerals, Cholesterol

INTRODUCTION

Nutrient requirements in broiler chickens change with age [1], growth performance with respect to the availability of basic nutrients like protein, carbohydrates, fats and minerals in the diet, as well as the source of protein. High protein diets are known to influence the growth performance of broilers by affecting the physiological mechanisms of the bone framework, fat conversion (soften tissues), and high hormonal levels [2]. Excessive or poor protein can be detrimental to health because it is associated with degenerative diseases such as obesity and cancer [3].

The addition of feed additives is a widespread practice to enhance the growth rate and feed conversion in broiler chickens [4]. Zinc bacitracin consumption has been reported to increase body weight of chicken by 10.8%, and reduce food conversion with a 31% increase in abdominal fat deposit in female chickens [5]. A study on Zn bacitracin treatment on broiler chicken, demonstrated that the addition of the antibiotic at 33mg or 55mg per kg diet improved growth rate and feed utilization efficiency [6], resulted in a higher frequency of tibial dyschondroplasia (TD) and a higher frequency of dust bathing [7].

Experiments have also shown that Zn bacitracin can significantly reduce the number of Coliform bacteria in the ileum and increase the activities of amylase and lipase in pancreas homogenates, thereby increasing the growth rate [8]. Antibiotics (avoparcine and tetracycline) may play a role in broiler growth depression relating to competition in nutrient uptake or impaired fat absorption due to bile acid deconjugation [8]

No adverse pathological changes were observed in the examined tissues, indicating that avoparcine was well tolerated [9].

Excess protein in the diet can increase risk of the animal or bird bacterial disease (*Brachyspira pilosicoli*) in broilers [10, 11]. The main site of antibiotic activity in a chicken is within the gastrointestinal tract, where Zn bacitracin acts to modify the intestinal flora as well as the gut wall structure, in order to channel more nutrients into the broiler's body/tissue and thus enhance growth [4].

Presently, the use of broad-spectrum antibiotics remains the most common cause of antibiotic resistant bacteria in poultry, cattle, calves, eggs, and milk. In the USA, over two-thirds of outbreaks of multiple drug resistant *Salmonella* infections come from food, transmitted to humans mainly through consumption of food produced from animal products containing traces of antibiotic/food additives [12]. Antibiotic resistance is not the only danger inherent in modern animal husbandry practice. *Bovine Spongiform Encephalopathy* is believed to have spread from Britain because of the practice of incorporating material from the carcasses of dead cattle and other animal products into the cattle feed, thereby resulting in people being infected after eating the beef from the infected cattle [13].

The role of growth hormones (GH) in the control of animal growth is well documented [14]. Growth hormone exhibits somatogenic or metabolic regulatory functions with regard to the post-absorptive use of nutrients and its use in animal production has focused on its influence on muscle to fat ratios to improve lean meat in response to exogenous administration. The anabolic effect of GH on muscle is presumed to be largely mediated by the insulin-like growth factor (IGF-I), which stimulates protein synthesis [15]. Testosterone in large doses inhibits pituitary activity in both male and female birds, suppressing ovulation and spermatogenesis [16]. In small physiological doses, testosterone supports spermatogenesis. In skeletal muscle it increases creatin storage, muscular development and endurance. Testosterone has a positive nitrogen balance and growth promotion effect on birds. In the nervous system, it improves resistance of the central nervous system to fatigue [16]. Higher level would certainly be detrimental to the health of the birds.

There has been little evidence on the role of growth promoter on the physiology of animals in relation to bone health and body mass. The optimum dietary mineral contents, particularly in relation to bone formation and impairs growth performance remain controversial [17]. In a study carried out by William, a slight tendency for higher weights was observed at low dietary Ca and higher percentage ash occurred at higher dietary Ca, but there was little evidence of conflict between bone mineralization and final body weight [18].

The major objective of this study was to identify the presence of lipids and hormones in blood plasma and (Ca) and (P) in bones in response to Zn bacitracin treatment of broilers birds fed diets with either animal or plant protein.

MATERIALS AND METHODS

Diets: Plant and animal protein diets were formulated using the standard computerized feed formulation programme [19]. 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 labeled.

Feeding trial: The feeding trial protocol used was battery "Cage system". Two feeding trials were conducted consecutively. In the first trial, 240 day-old chicks were divided into four (4) groups, and in the second trial, 120 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^{o}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 and growth parameters were pooled.

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 300gm per tonne

Table 2: Composition of plant and animal diets for broiler chickens

RAW MATERIALS	PLANT PROTEIN DIETS (KG)	NUTRIENTS	(Percentage) %
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	(Percentage) %
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.

The birds were fed daily at 9.00am, and both feed and water were provided *ad-libitum* [20]. 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 [20].

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, 6000rpm) and the plasma was stored at –15°C. Total cholesterol, HDL-cholesterol, LDL –cholesterol, and triglyceride levels were determinated by Chemical pathology routine Diagnostic laboratory at Groote Schuur Hospital situated in Cape Town, South Africa [21].

The plasma hormonal analyses were carried out to determine; oestradiol, progesterone, and testosterone levels in broiler chickens with and/ or without the inclusion of Zn bacitracin. The progesterone levels were determined using RIA. The Coat-A-Count Progesterone kit ^{125}I radioimmunoassay designed for the direct, quantitative measurement of progesterone in plasma was used (Cat. No. TKPG1supplier Diagnostic Products Corporation,). The testosterone levels were determined using Chemiluminescence System; the Automated Chemiluminescence System was selected. ACS: 180 kits for Testosterone +E (Cat. No. 672324 manufactured by Chiron Diagnostics and supplied by Bayer). The oestradiol levels were determined using in-house assay while the extraction and assay procedures were modifications of Chemical Pathology lab at UCT Medical School, Groote Schuur Hospital [22]. All plasma samples were thawed and allowed to come to reach room temperature before analyzing for testosterone and progesterone. Samples collected in the first run of the experiment were also analyzed for oestradiol [23].

The determination of Ca and P content in the bones and claws was done after slaughtering and were stored at –15°C. All tissues were manually removed from the bones, the bones and claws were dried in an oven 80 - 120°C for 24hrs. The bone was weighed to the nearest 0.1mg, ashed in a Muffle furnace at 500°C for 12hrs, and ash weight determined to the nearest 0.1mg [24].

Ca and P of the bone were determined using the Nitrious Oxide/acetylene flame of a Atomic Absorption-spectrophometer, after standard acid bone digestion using the technique described by Moor and Chapman [24].

STATISTICAL ANALYSES

The Data was subjected to Repeated measures ANOVA, using the Levene's Test of Homogeneity of Variances, of the Statistical Analysis system [25].

RESULTS

The dietary regimes significantly affected the Ca and P levels in thighbones. Ca levels in broilers on the plant protein regime (treatment A) had significantly higher ($p<0.05$) Ca/g dry bone than all other treatments. The average value of Ca in thighbones was 292.1mg Ca/g dry bone. The addition of Zn bacitracin as in treatment C resulted in a significantly decrease ($p<0.05$) in Ca to 262.2mg Ca/g dry bone. The thighbones of broilers on treatment B (animal based diet) showed an average increased Ca level of 263.6mg Ca/g dry bone and on addition of Zn bacitracin (treatment D), there was a decrease to (257.5mg Ca/g) dry bone as shown in (Fig. 1). P levels did not differ between treatments ($p>0.05$).

Table 3: Calcium and Phosphorus levels in Thighbones of broilers

Treatments	Thighbones	Thighbones
	Ca (mg/g)	P (mg/g)
A	292.05	2.38
B	262.63	225
C	262.16	2.21
D	257.46	2.21

Mean mg/g of Calcium and Phosphorus levels in thighbones in treatments (A, B, C and D)

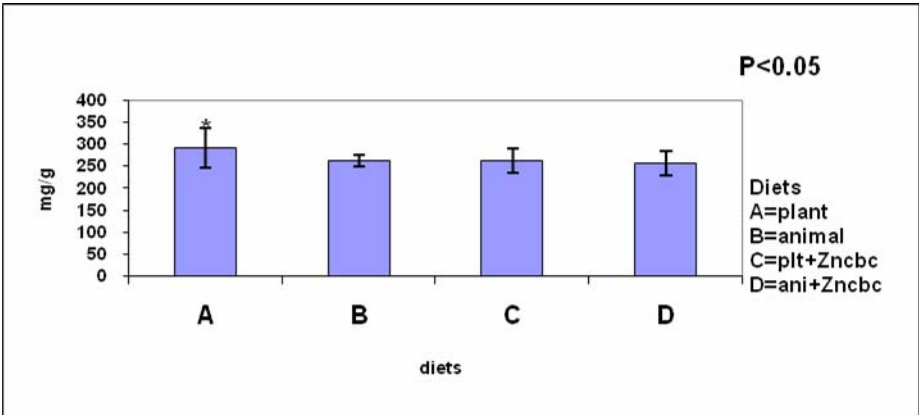

Figure 1: Mg/g Ca in thighbone of broilers receiving different dietary regimes.

Weight (mg/g Ca) of left thighbones of broiler chickens fed with different dietary regimes are as follow: plant-based protein source (A), animal-based protein source (B), plant-based protein supplemented with Zinc bacitracin (C) and animal-basedprotein supplemented with Zinc bacitracin (D) * = (P<0.05) significantly different.

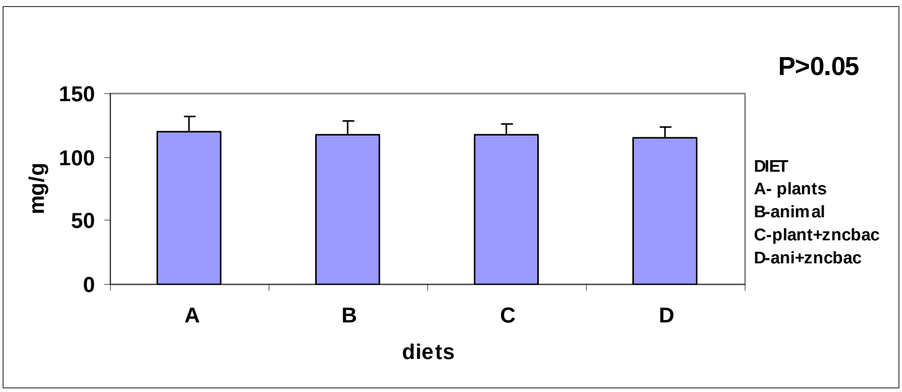

Figure 2: Mg/g P in thigh bone of broilers receiving different dietary regimes.

Weight (mg/g P) of left thighbones of broiler chickens fed with different dietary regimes are as follow: 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).

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 3). Treatment B (Animal protein diet without Zn bacitracin) produced the highest total cholesterol level, followed by D, A, C.

Table 4: 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).

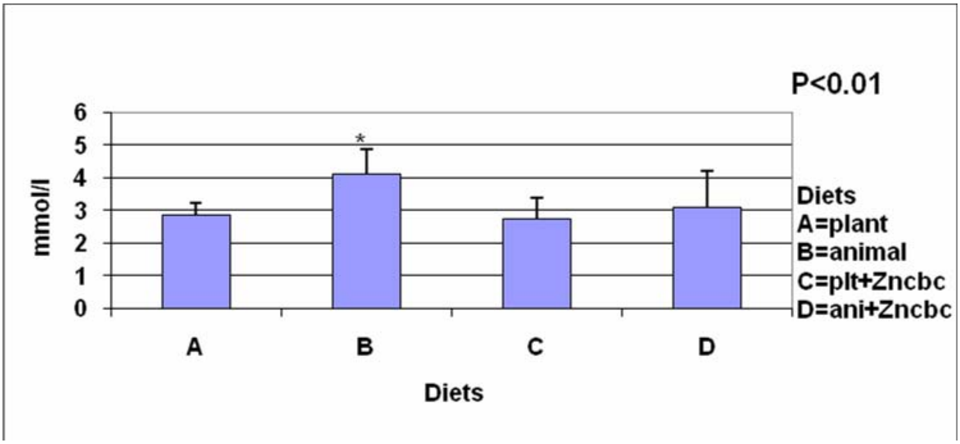

Figure 3: Total Cholesterol in plasma of broiler chicken feed different dietary regime.
Mean plasma total cholesterol levels of broiler chickens fed with different dietary regimes are as follows: plant-based protein source (A), animal-based protein source (B), plant-based protein supplemented with Zinc bacitracin (C) and animal-based protein supplemented with Zinc bacitracin (D) * = (P<0.05) significantly different. * = (P<0.05) significantly different.

Regime (B) showed significant ($p<0.05$) 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 2.55mmol/l, representing slightly higher levels than human standard of (0.7 - 2.5mmol/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 - 2.5mmol/l). Treatments (A, C and D) showed HDL value (0.7 – 2.5mmol/l), which are within the average human standards (Fig.4).

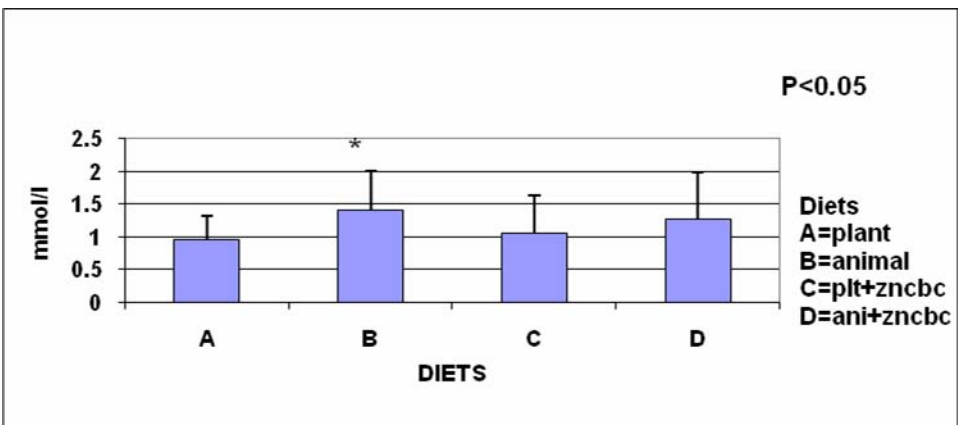

Figure 4: Mean HDL plasma levels in broiler chicken feed different dietary regime.
Mean plasma HDL levels of broiler chickens fed with different dietary regimes are as follows: plant-based protein source (A), animal-based protein source (B), plant- based protein supplemented with Zinc bacitracin (C) and animal-based protein supplemented with Zinc bacitracin (D) * = (P<0.05) significantly different. * = (P<0.05) significantly different.

The plasma LDL 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 - 3.5mmol/l). Treatment (B) animal protein diet showed the highest value of (1.41mmol/l), followed by treatment D (1.26mmol/l), which is when Zn bacitracin is added to the animal protein diet (B) while in plant protein diet (A) the value of (0.90mmol/l) was increased on addition of Zn bacitracin (C) to (1.00mmol/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) (Figures 5 and 6).

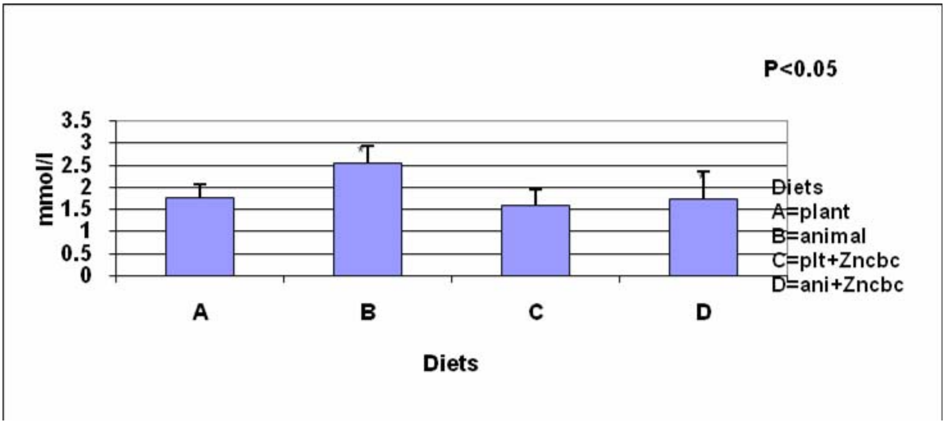

Figure 5: Mean plasma LDL levels in broilers receiving different dietary regimes
Mean plasma LDL levels of broiler chickens fed with different dietary regimes are as follows: plant-based protein source (A), animal-based protein source (B), plant-based protein supplemented with Zinc bacitracin (C) and animal-based protein supplemented with Zinc bacitracin (D) * = (P<0.05) significantly different. * = (P<0.05) significantly different.

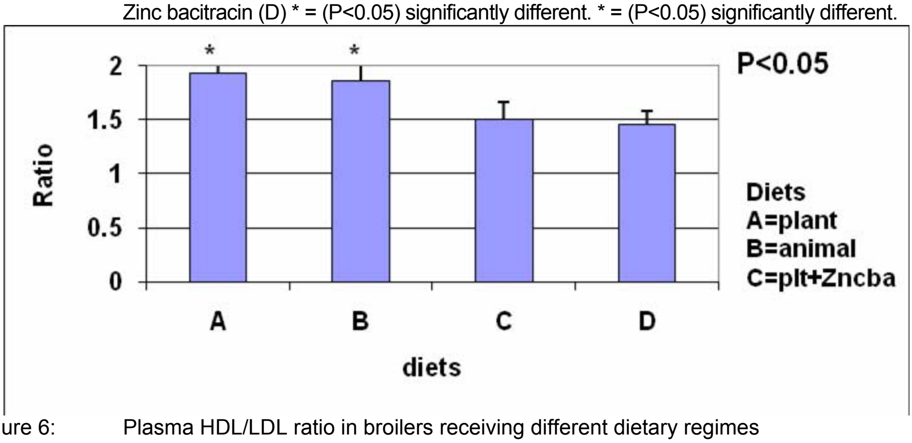

Figure 6: Plasma HDL/LDL ratio in broilers receiving different dietary regimes
Mean plasma HDL/LDL levels of broiler chickens fed with different dietary regimes are as follows: plant-based protein source (A), animal-based protein source (B), plant-based protein supplemented with Zinc bacitracin (C) and animal-based protein supplemented with Zinc bacitracin (D) * = (P<0.05) significantly different.

Oestrogen levels were similar to human standard ranges (50 –2000 pmol/l for blood and 50 – 2000 pmol/g for tissue). Broiler chickens fed with plant protein plasma (A), showed the highest levels of oestrogen, but a significantly lowered result was obtained when Zn bacitracin was added (C) (Fig 7). Similar results were observed in animal protein feeding regimes. Progesterone plasma analysis, showed similar trends to oestrogen, in which high plasma progesterone levels was observed in broiler chickens fed with plant protein, but the addition of Zinc bacitracin lowered the levels of progesterone significantly (p<0.05).

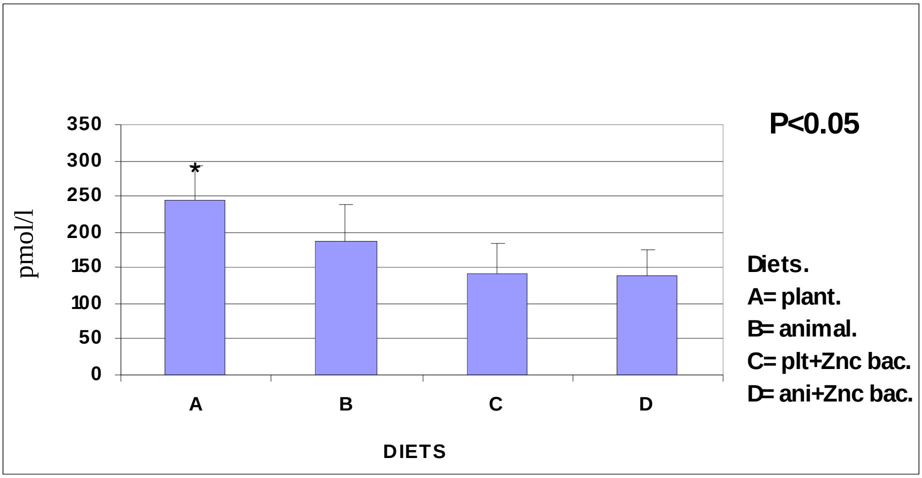

Figure 7: Plasma Oestrogen levels in broilers receiving different dietary regimes. Mean plasma Oestrogen levels of broiler chickens fed with different dietary regimes are as follows: plant-based protein source (A), animal-based protein source (B), plant-based protein supplemented with Zinc bacitracin (C) and animal-based protein supplemented with Zinc bacitracin (D) * = (P<0.05) significantly different.

The highest level of progesterone was recorded in treatment A (2.31nmol/l), and this was significantly higher (P<0.05) than other treatments. However, the addition of Zn bacitracin to plant protein diet (C) resulted in a lowered progesterone level (0.96nmol/l). Treatment B, involving animal protein diet with an average value of 1.45nmol/l, was also slightly below that of the standard human value and was also reduced when Zn bacitracin was added (treatment D animal protein diet + Zn bacitracin). This value 1.02 nmol/l is also lower than those of the human value (Fig. 8).

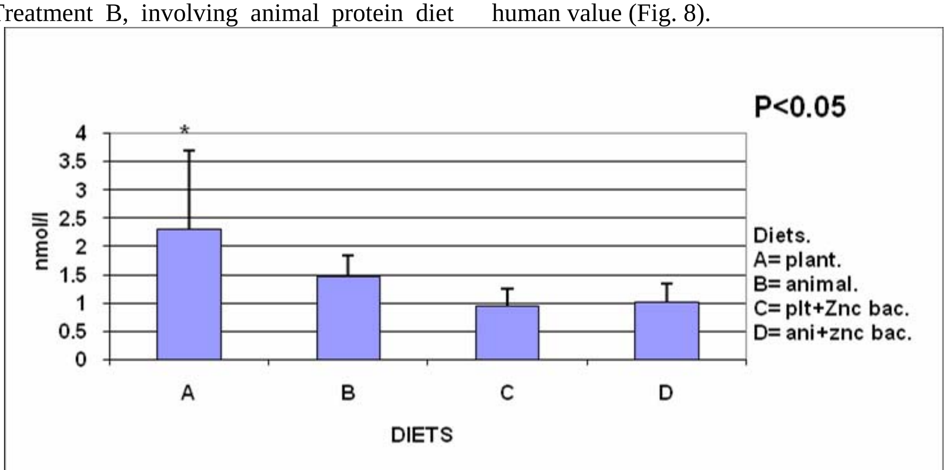

Figure 8: Plasma progesterone levels in broilers receiving different dietary regimes. Mean plasma progesterone levels of broiler chickens fed with different dietary regimes are as follows: plant-based protein source (A), animal-based protein source (B), plant-based protein supplemented with Zinc bacitracin (C) and animal-based protein supplemented with Zinc bacitracin (D) * = (P<0.05) significantly different.

Testosterone showed non significant differences ($P>0.05$) between treatments (A, B, C, D), and values ranged from 0.46 – 1.14nmol/l fall within human standard ranged of 0.5 – 2.7nmol/l.

DISCUSSION

High protein influences growth performance through the bone frame work, fat conversion (soft tissues), and high hormonal levels, while antibiotic growth promoter Zn bacitracin will reduces Ca levels in broilers fed plant protein diets [6]. There was a significant difference ($p<0.05$) in bone Ca between treatments, P did not differ. The average bone Ca:P ratio for the plant-based diet without Zn bacitracin (treatment A) was higher than any other treatments (B, C, D). This indicates that broiler chickens on plant protein diet are more efficient in incorporating Ca. This is comparable to the result of the research carried out in sheep, were more Ca was deposited in bones when plant protein diets were fed compared to animal protein diets [26].

Dietary animal protein severely compromised bone development, although weight gain and food consumption did not differ from plant protein [26, 27]. Another study on sub-therapeutic Zn bacitracin showed effects on bile acid-transforming enzymes in small-intestinal homogenates [28]. The inverse relationship between growth performance and cholyltaurine hydrolase activity raises the possibility that specific inhibitors of this enzyme may promote weight gain and feed conversion in livestock, thereby reduce or eliminate the need for antibiotic feed additives [28].

One of the 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 [29]. A ratio favoring 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 [3, 29]. Inclusion of Zn bacitracin enhances overall growth, thereby affecting overall metabolism in the broiler chickens. In cholesterol channeling 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 (3). 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 [30, 31, 32].

High oestrogen levels was observed in broiler chickens fed plant protein diets, and this falls when Zn bacitracin was added, an indication that oestrogen might have been deposited in tissues of broilers. This may be associated with cancer [33 and 16] in humans due to potent stimulation of cell growth. High dietary intake of steroid hormones can increase the risk of cancer to consumers [33]. It can be assumed that oestrogen levels in broiler meat might be slightly higher than in plasma when Zn bacitracin is added, and thus could contribute to overall oestrogen load especially in regular consumers. This finding is important, as similar reduction can be observed in progesterone concentration in plasma when Zn bacitracin is added, an indication of tissue deposition.

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 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.

In view of the effects of oestrogen with regards to reproductive problems, carcinogenicity and obesity, Zn bacitracin could be considered a contributing factor to increased toxic levels of hormone if the broiler chickens are fed plant protein diets with Zn bacitracin. Plant protein diets without Zn bacitracin produced the lowest levels of oestrogen, and should be promoted for broiler chicken feed.

Feeding broiler chickens with growth promoters, as well as animal protein and Ca sources such as poultry by-products comprising of; feathers, offal' and dead birds, fish meal, carcass meal, may be economically viable, but this is an acid forming diet, thus it will take its toll in terms of having a negative impact on mineral utilization and incorporation into bone tissue.

In view of the short maturation period of broiler chicken (about six weeks), appropriate time should be given to the birds for possible elimination of the drugs and its by products from the chicken metabolism, before considering the birds ready for market, even though this may not be a major issue, policies can be geared towards either a complete stoppage of the use of these kinds of drugs, or the reduction of its usage.

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