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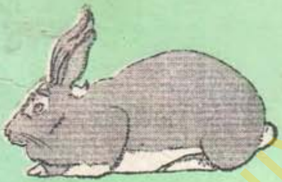
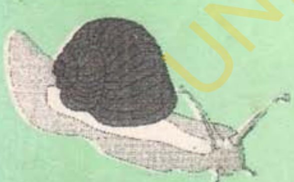


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EFFECTS OF DIETARY ACIDIFER-BASED DIET ON HAEMATOLOGY AND SERUM BIOCHEMICAL INDICES OF BROILERS

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ABSTRACT

A trial was conducted to determine the effect of dietary Biotronics SE® supplementation on haematological and serum parameters of broilers. A total of 120 1-day old Marshall broilers were randomly distributed to four dietary treatments using completely randomized design. Chicks in treatment 1 were fed basal diet (control) while those on treatments 2, 3 and 4 were given basal diets supplemented with 0.1% oxytetracycline HCl only; 0.3% Biotronics SE® only and 0.1% oxytetracycline HCl + 0.3% Biotronics SE®, respectively. Each treatment was a duplicate of fifteen chicks per replicate. The diets were formulated to contain 3000 Kcal/kg ME and crude protein of 23% (starter) and 20% (finishers). Variations in values obtained for haemoglobin (g/dl) (9.28, 9.69, 10.04 and 10.15) and Mean Cell Volume (MCV) (fl) (107.02, 109.38, 125.43 and 118.31) for birds fed diets 1, 2, 3 and 4., respectively were significantly ($p<0.05$) different while other haematological parameters measured in both phases were similar ($p>0.05$). The serum triglycerides (mg/dl)(23.19, 19.29, 23.93 and 19.01) and VLDL (mg/dl) (4.63, 3.85, 5.78 and 3.80)for the starter birds fed diets 1, 2, 3 and 4, respectively were affected by dietary acidifier inclusion. The serum total proteins and cholesterol also varied significantly for finishers birds. Generally, most serum indices measured declined in values at the finishers phase compared with corresponding values for starter. All measured indices were within normal physiological ranges. This study revealed Biotronics SE® as a suitable alternative growth promoter for the production of meat type chickens

Key words: Acidifier, Haematology, Serum, Broilers, Antibiotics growth promoters

INTRODUCTION

Growth Promoting Antibiotics (GPAs) have been widely used in animal production for the last fifty years. The practice of feeding GPAs has been well-established and become an integral part of nutritional strategies for commercial farm animals (Dibner and Richards, 2005). Feeding Broilers with GPAs supplemented diets have been reported to increase average daily weight gain and improved feed efficiency by 3-8% and 3%, respectively (Kim *et al.*, 2005). Fairchild *et al.* (2001) reported an improvement in health and performance of poultry birds through reduction in the population of pathogenic microbes present in the gastro intestinal tract. Throughout the developed world, there has been increasing public and governmental concerns about increasing prevalence of resistance to antibiotics in disease causing bacteria. There are concerns that many antibiotics currently available to treat human diseases will no longer be effective (Casewell *et al.*, 2003). According to Wary and Davis (2003), the spread of antibiotic resistant bacteria as residues in consumed animal products are threat to human health. This negates the promotion of safe animal and animal products as emphasized by the World Health Organization (WHO). In response to safe, healthy animal and animal products devoid of GPA-resistant bacteria residues, the European Union in 2002 banned the use of GPAs in livestock production.

The ban on GPA necessitated the search for alternative feed additives to alleviate the problems associated with the withdrawal of antibiotics from animal feed. Among the potential replacements for antibiotics are organic acids which are referred to as acidifiers (Sterner, 2006). In livestock production, weak organic acids have been used for preservation of feed against fungi and bacteria contamination. Kirchgessner and Roth (1998) concluded that feed acidification with formic, propionic, lactic, acetic, citric acids and their corresponding salts in broiler diets decreased colonization of gut by pathogenic microbes, improved digestibility of proteins and some major minerals. According to Partanen (2001), the ability of organic acids to replace antibiotics is centered around its mode of action, which is based on their specific ability to penetrate the bacteria cell wall and kill bacteria by interfering with their metabolism. The ions in organic acids pass through bacteria cells intact and undissociated. Senkoylu *et al.* (2007) reported that a mixture of organic acids as dietary acidifiers not only broadened their spectrum of action and also an ensured combined effect of the good qualities of the different acids.

The ingestion of numerous dietary components has measurable effects on blood constituents, (Church *et al.*, 1984). Blood with its myriad of constituents provides a valuable medium for both clinical investigations and nutritional evaluation of the organisms (Aderemi, 2004). In spite of the growth promoting and gut managing potentials of dietary acidifiers little is known about its impact on the blood picture which depicts the physiological conditions of the organs as well as the health status of the birds. The purpose of this study was to examine the haematological and selected serum biochemical indices of broiler fed acidifier supplemented diets.

MATERIALS AND METHODS

Experimental Animals

A total of one hundred and twenty day-old Marshal Broiler chicks were obtained from a reputable commercial hatchery in Ibadan. Routine management, medication and vaccination were adopted as described by Oluyemi and Roberts, 2000. The birds were thereafter randomly allotted equally to four dietary treatments of thirty chicks per treatment and brooded on partitioned deep litter house at the University of Ibadan Teaching and Research Farm. Each treatment was a duplicate of fifteen chicks each. Four isocaloric and isonitrogenous experimental diets were formulated at the starter and finishers' phases as shown in Tables 1 and 2.

Diet 1 - (Control Diet) - without Oxytetracycline HCl and Biotronics SE®

Diet 2- (Control Diet + 0.1% Oxytetracycline HCl)

Diet 3- (Control diet + 0.3% Biotronics SE®

Diet 4- (Control diet + 0.1% Oxytetracycline HCl + 0.3% Biotronics SE®

Each experimental diet was assigned to corresponding chicks. Feed in mash form and water were given *ad libitum* from day 1 to 21(Starter) and day 22 to 49 (Finishers').

Table 1: Composition of acidifier supplemented Starter diets (g/100g)

Ingredients	Dietary treatments 1	2	3	4
Maize	50.00	50.00	50.00	50.00
Full fat soya	35.00	35.00	35.00	35.00
Brewers dried grain	9.97	9.87	9.67	9.57
Palm oil	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.50	1.50	1.50	1.50
Common salt	0.30	0.30	0.30	0.30
L-Lysine	0.07	0.07	0.07	0.07
DL Methionine	0.16	0.16	0.16	0.16
Mycofix Select	0.20	0.20	0.20	0.20
Avatec	0.06	0.06	0.06	0.06
Broiler Starter Premix*	0.25	0.25	0.25	0.25
Oyster shell	0.05	0.05	0.05	0.05
Oxytetracycline-HCl	-	0.10	-	0.10
⁺Biotronics SE®	-	-	0.30	0.30
Nutrient density				
Crude Protein (%)	22.01	19.99	19.93	19.90
ME (Kcal/kg)	3240	3238	3233	3230

Each 2.5 kg of Nutrivitas/DSM Nutripoults Broiler vitamin/mineral premix contains: Vitamin A 10,000,000 iu, vitamin D_3 2,000,000 iu, vitamin E 40,000 mg, Vitamin K_3 2,000 mg, vitamin B_1 1,500 mg, vitamin B_2 5,000 mg Vitamin B_6 4,000 mg, vitamin B_{12} 20 mg, Niacin 40,000 mg, Calpan 10,000 mg, folic acid 1000mg, biotin 100 mg, Antioxidant 100,000 mg, choline chloride 300,000 mg, Manganese 80,000 mg, Iron 40,000 mg, Zinc 60,000 mg, copper 80,000 mg, Iodine 80,000 mg, cobalt 300mg, selenium 200 mg.

⁺ *(17.4% Formic acid, 14.1% Ammonium Formate, 12.4% Propionic acid, 8.4% Ammonium Propionate and 47.7% filled material(Fructo-oligosaccharide, as the carrier)*

Table 2: Composition of acidifier supplemented finishers' diets (g/100g)

Ingredients	Dietary treatments 1	2	3	4
Maize	50.00	50.00	50.00	50.00
Full Fat Soya	30.00	30.00	30.00	30.00
Brewers Dried Grain	9.97	9.87	9.67	9.57
Palm oil	1.89	1.89	1.89	1.89
Dicalcium Phosphate	1.50	1.50	1.50	1.50
Common Salt (NaCl)	0.30	0.30	0.30	0.30
L-Lysine	0.06	0.06	0.06	0.06
DL Methionine	0.14	0.14	0.14	0.14
Mycofix Select	0.20	0.20	0.20	0.20
Avatec	0.06	0.06	0.06	0.06
Broiler Starter Premix*	0.25	0.25	0.25	0.25
Oyster shell	0.05	0.05	0.05	0.05
Oxytetracycline HCl	-	0.10	-	0.10
⁺Biotronics SE®	-	-	0.30	0.30
Nutrient density (Cal)				
Crude Protein (%)	22.01	19.99	19.93	19.90
Metabolizable Energy (Kcal/Kg)	3240	3238	3233	3230

Each 2.5Kg of Nutrivitas/DSM Nutripoults Broiler vitamin- Mineral premix contains: Vitamin A 10,000,000 iu, vitamin D $_2$, 1000,000 iu, vitamin E 40,000 mg, Vitamin K_3 2,000 mg, vitaminB_1 1,500 mg, vitaminB_2 5,000 mg Vitamin B_6 4,000 mg, vitamin B_{12} 20 mg, Niacin 40,000 mg, Calpan 10,000 mg, folic acid 1000mg, biotin 100 mg, Antioxidant 100,000 mg, choline chloride 300,000 mg, Manganese 80,000 mg, Iron 40,000 mg, Zinc 60,000 mg, copper 80,000 mg, Iodine 80,000 mg, cobalt 300 mg, selenium 200 mg.

⁺ *(17.4% Formic acid, 14.1% Ammonium Formate, 12.4% Propionic acid, 8.4% Ammonium propionate and 47.7% filler (Fructo-oligosaccharide, as the carrier)*

Blood Samples Collection

Blood samples were obtained from the jugular vein of four birds per replicate at day 21 (starter phase) and 49 (finishers' phase) in sterile Vacutainer tubes containing EDTA (an anticoagulant) for haematology, while that for serum indices was without EDTA. Blood samples for serum assay were centrifuged and serum was separated and stored at -10^0 C.

Haematology
The Packed Cell Volume (PCV) and Hemoglobin (Hb) were determined using micro-haematocrit method and cyano met haemoglobin method, respectively (Mitruka and Rawnsley, 1977). Erythrocyte count (RBC) and Leukocyte Count (WBC) were determined using the improved Neubauer haemocytometer after the appropriate dilution (Schalm *et al.*, 1975).

Serum indices
Glucose was determined by the O-Toluidine method using acetic acids (Cooper and McDaniel, 1970).Total serum protein was determined using the Buiret method (Kohn and Allen, 1995). Albumin was determined using the BCG (Bromocersol green) method as described by Peter *et al.* (1982). Globulin was obtained by subtracting albumin from total protein. Serum cholesterol and triglycerides were measured using appropriate labouratory kits (Friedwald *et al.*, 1972; Gowenlock *et al.*, 1988). Very Low Density Lipoprotein (VLDL) cholesterol was calculated from triglycerides by division with 5.

Data Analysis
Data were subjected to one way analysis of variance for completely randomized design (SAS, 1999).

RESULTS AND DISCUSSION

Haematological indices Haematological parameters of broilers fed acidifier supplemented diets at both starter (0-4 weeks) and finishers' (4-7weeks) phases are presented in Tables 3 and 4. Values obtained for the PCV% of birds at the starter phase (28.00, 28.62, 30.00 and 30.50 for birds on diets 1, 2, 3 and 4 respectively) were not significantly ($p > 0.05$) affected by dietary treatments. At the finishers phase, similar non significant ($p>0.05$) variations were obtained for PCV% values which were lower for birds on treatments 1 (28.25%) and 2 (28.75%) compared to those on treatments 3 (31.25%) and 4 (31.25%). However the values obtained were within reported reference range of 22.00-35.00% by (Jain, 1993) and 28.00-37.00% (Clinical Diagnostic Division, 1990).

Values obtained for Hb (g/dl) at the starter phase were 9.28, 9.69, 10.04 and 10.15 for birds on diets 1, 2, 3 and 4 respectively. This index was significantly ($P<0.05$) higher for birds on treatment 4. On the other hand, statistically similar ($p>0.05$) values of 10.42, 10.42, 9.40 and 9.60 were obtained for birds on treatments 1, 2, 3 and 4 respectively at the finishers' phase. However the values obtained were within the normal physiological range (Mitruka and Rawnsley, 1977). The values obtained for RBC ($x10^6mm^3$) ranged between 2.47-2.69 at the starter phase and 2.49-2.64 at the finishers phase. The variations in the values were not significantly ($p>0.05$) different. All values obtained for RBC conformed to the reference range as reported by Mitruka and Rawnsley (1977). This suggested that dietary supplementation with acidifier did not antagonize haemopoiesis through the inhibition of protein digestion or abnormality in iron absorption for the synthesis of haeme.

The values obtained for MCHC% and WBC ($x10^3mm^3$) of birds fed diets 1 2, 3 and 4 at both the starter and the finishers' phases were statistically similar ($p>0.05$). Although mean haemoglobin content of birds on diet 4 was statistically ($p<0.05$) different from that of control. No variation was observed when compared with birds fed diets 2 and 3, at the starter phase. At (4-7weeks), dietary treatments had no significant effect on haemoglobin content of the birds. For MCV (*fl*), dietary treatments significantly ($p<0.05$) affected the values obtained for the birds at the starter phase. The MCV (*fl*) of birds on diet 3 (125.43) and 4 (118.31) were significantly higher than the values of 107.02 and 109.38 obtained for birds on treatments 1 and 2. The higher values of MCV (*fl*) for birds on diets 2, 3 and 4 suggested that growth promotion activities of the test ingredients might cause increases in size of RBC. Increased size of erythrocytes could be an indication of megalobastic anaemia, the values obtained were within normal reported range 90-140 *fl* (Jain, 1993). At finishers' stage, dietary treatments had no in*fl*uence on the value of MCV. However the increased values obtained for birds on diets 2, 3 and 4 at both phases could be due to effect of feed additive on cell volume. Mean MCHC values for broilers across dietary treatments in both phases were not affected by dietary treatments. This observation could be attributed to the adequacy in haemoglobin content of the blood, suggesting that both acidifiers and oxytetracycline did not impede oxygen the carrying capacity of the blood in meeting the demand of the broilers.

The WBC value of birds varied numerically with higher values observed for starters compared to finishers. Birds on Diets 2 and 3 had higher Leukocyte than the control at (0-4weeks).This observation might probably be due to immune moderating characteristics of GPA in young chick, through their sub-therapeutic mode of action. Values of haematological indices obtained for birds at both phases of the assay were in harmony with those reported values (Mitruka and Rawnsley, 1977; Clinical Diagnostics Division, 1990; Jain, 1993).

The observable healthy status of the experimental birds agreed with the findings of Akinyele *et al.* (2009) that dietary GPA had no adverse effect on haematology of broilers. Onifade and Odunsi (1993) also reported similar finding that all haematological and erythrocytic indices of broiler chickens were not affected by Procaine Penicillin supplementation when the chickens were fed low and high fibre (BDG) based diets. Sarinee *et al.* (2008) in their study surmised that supplementation of chlortetracycline and probiotics in the diets of broilers had no effect on their antibody titres and haematological profile.

Table 3: Haematological indices of broilers fed acidifier supplemented starter diets

Parameters	Treatments				
	1	2	3	4	SEM
PCV (%)	28.00	28.63	30.00	30.50	1.16
Haemoglobin (g/dl)	9.28^{b}	9.69^{ab}	10.04^{ab}	10.15^{a}	0.38
RBC($x10^{6}/mm^{3}$)	2.65	2.69	2.47	2.61	0.16
MCV (*fl*)	107.02^{b}	109.38^{b}	125.43^{a}	118.31^{ab}	4.14
MCHC (%)	33.14	33.70	33.15	34.24	1.33
WBC($x10^{3}/mm^{3}$)	30.15	31.12	31.40	28.40	1.53

Means in the same row with different superscript differs significantly (P<0.05)
SEM=Standard error of the mean. PCV-Packed cell volume, RBC-Red Blood Cells, WBC-White Blood Cells, MCV-Mean Corpuscular Volume, and MCHC- Mean Corpuscular Haemoglobin Concentration

Table 4: Haematological indices of Broiler fed Experimental Finishers 'Diets

Parameters	Treatments				
	1	2	3	4	SEM
PCV (%)	28.25	28.75	31.25	31.25	3.52
Haemoglobin(g/dl)	10.42	10.42	9.40	9.60	0.58
RBC ($x10^{6}/mm^{3}$)	2.53	2.49	2.64	2.52	0.07
MCV (*fl*)	109.74	119.06	118.39	121.00	5.84
MCHC (%)	33.09	33.40	32.68	33.60	0.84
WBC ($x10^{3}/mm^{3}$)	23.95	20.80	21.15	22.55	1.80

Means in the same row with different superscript differs significantly (P<0.05).
SEM=Standard error of the mean; PCV-Packed cell volume, RBC-Red Blood Cells, WBC-White Blood Cells, MCV-Mean Corpuscular Volume, and MCHC- Mean Corpuscular Haemoglobin Concentration

Serum biochemical profile

Results of selected serum biochemical indices of broilers fed acidifier supplemented diets at both starter and finishers' phases are presented in Tables 5 and 6. The statistically similar (p>0.05) mean serum total proteins (g/dl) obtained were 3.39, 2.91, 3.18 and 3.30 for birds on starter diets 1, 2, 3 and 4, respectively. Corresponding statistically significant P<0.05) values obtained for birds on finishers diets 1, 2, 3, and 4 were 3.24, 3.21, 3.02 and 4.59 respectively. Birds on treatment 4 recorded persistently higher levels of serum total proteins both at the starter and finishers' phases. The combination of the two additives probably enhanced protein synthesis and utilization in the experimental birds. The variations in the mean serum globulin values obtained for birds at both phases were statistically similar (p>0.05). However, values at the finishers' phase were higher than those at the starter'. These higher values might be attributed to increased synthesis of gamma globulin which participates in immunological reactions. Since the immune system gradually mature with ageing, it is to be expected that the quantity of gamma globulin fraction in birds increased consequentially. Losonczy *et al.*, (1972) had similar observation using geese in his study noted that concentration of antibodies increased up to the age of two months after which they remained constant.

Dietary treatments did not affect the serum glucose concentration of the birds at both phases. Age is a factor in the higher blood glucose values observed for chicks at starter phase which conformed earlier observation (Adeyemo and Longe, 2007) that age significantly affects utilization of nutrients with higher digestibility and nutrient retention, the hall mark at early stage of growth when the birds are actively building tissues. Hernandez, *et al.* (2006), also reported that dietary supplementation of Avilamycin, and varying levels with formic acids had no significant effect on serum glucose of broilers. This showed that dietary acidifiers neither impede carbohydrate metabolism nor alter metabolic activities of liver in glucose uptake and hormonal action of insulin in glycolytic pathway. This also confirmed the assertion of Sheikh adil *et al.* (2004) that feeding broilers graded levels (1-3%) of different organic acids for (0-42days) had no significant in*fl*uence on serum glucose

The values obtained for serum cholesterol (mg/dl) (123.81, 137.90, 137.47 and 131.30) at the starter phase (for birds on treatments 1, 2, 3 and 4, respectively) were not affected (p>0.05) by dietary inclusion of acidifiers. Corresponding significantly varied (p<0.05) values for finishers' were 111.96, 140.72, 124.13 and 114.70 for birds on diets 1, 2, 3 and 4 respectively. Cholesterol in the body of animals is derived from both dietary sources and *de novo* synthesis in the liver and other tissues. The role of dietary additives in the binding of cholesterol in the gut causing it to be excreted in the faeces rather than being reabsorbed has been documented (Cheeke and Dierenfeld, 2010). Hernandez *et al*, (2006) concluded that feeding graded levels of formic acids to broiler chickens for 42 days had no effect on serum cholesterol metabolite. Feed additives can affect lipid and hepatic metabolism resulting in increased cholesterol (Murwani and Bayuardhi, 2007) However, values obtained conformed to normal physiological ranges reported by (Merck, 1999; Mitruka and Rawnsley, 1977).

Triglycerides which are also present in blood plasma and in association with Cholesterol form the plasma lipids. At the starter phase, serum triglycerides (mg/dl) values (23.19, 19.29, 23.93 and 19.01 for birds on diets 1, 2, 3 and 4, respectively) were significantly different (P<0.05). The corresponding values (16.61, 13.61, 11.91 and 15.49 for birds fed diets 1, 2, 3 and 4, respectively) were lower and statistically similar (p>0.05) in finishers. Similar trend occurred in the values of VLDL which at the starter were (4.63, 3.85, 5.78 and 3.80 for birds fed 1, 2, 3 and 4, respectively) significantly affected by the dietary inclusion of acidifiers. These values (3.32, 2.72, 2.38 and 3.10) declined and became statistically similar at the finishers phase. White and Griffin (1982) have correlated serum VLDL concentrations in fully-fed broilers with body fat content, which could act as an indirect measure of fatness in live birds. The use of additives in the modulation and mitigation of lipogenesis and lipid metabolism have been reported (Cheeke and Dierenfeld, 2010) and this factor could be relevant considering the fact that though lipid indices declined in the finishers generally compared to starters, the striking reduction in the VLDL and triglycerides components the finishers' fed acidified diets compared to others (control and oxytetracycline diets) has serious economic and health implications vis a vis contemporary crave and desire for meat of low fat

Table 5: Selected serum indices of broilers fed acidifier supplemented starter diets

Parameters	Treatments				
	1	2	3	4	SEM
Total Protein (g/dl)	3.39	2.91	3.18	3.30	0.22
Albumin (g/dl)	2.80^{a}	2.26^{b}	2.50^{ab}	2.48^{ab}	0.18
Globulin (g/dl)	0.57	0.64	0.61	0.89	0.26
Glucose (mg/dl)	210.31	234.11	231.63	224.89	10.97
Cholesterol (mg/dl)	123.81	137.90	137.47	131.30	13.63
Triglycerides (mg/dl)	23.19^{ab}	19.29^{b}	23.93^{a}	19.01^{b}	4.26
VLDL (mg/dl)	4.63^{ab}	3.85^{b}	5.78^{a}	3.80^{b}	0.85

Means in the same with different superscript differ significantly (P<0.05). SEM=standard error of the mean; VLDL (Very Low Density Lipoprotein Cholesterol)

Table 6: Serum Biochemical indices of broilers fed acidifier supplemented finishers' diets

Parameters	Treatments				
	1	2	3	4	SEM
Total Protein(g/dl)	3.24^{b}	3.21^{b}	3.02^{b}	4.59^{a}	0.39
Albumin(g/dl)	2.23	2.42	1.78	2.62	0.29
Globulin(g/dl)	1.00	0.77	1.22	1.97	0.43
Glucose(mg/dl)	160.77	207.26	192.39	179.28	26.63
Cholesterol(mg/dl)	111.96^{a}	140.72^{a}	124.13^{a}	114.70^{b}	9.59
Triglycerides(mg/dl)	16.61	13.61	11.91	15.49	2.11
VLDL(mg/dl)	3.32	2.72	2.38	3.10	0.42

Means in the same with different superscript differ significantly (P<0.05).

SEM=standard error of the mean VLDL (Very Low Density Lipoprotein. Cholesterol)

CONCLUSION

The growth promoting action of acidifiers and their modulating effects in the gastro intestinal tracts had no consequence on the gross profile of blood and health of the birds. Therefore the use of dietary acidifiers as alternative feed additive particularly in the moderation of meat lipid content should be fully assessed and exploited to replace antibiotics in broilers nutrition.

REFERENCES

Aderemi, F. A. 2004. Effects of replacement of wheat bran with cassava root sieviate supplemented or unsupplemented with enzyme on the Haematology and Serum biochemistry of pullet chicks. *Trop. J. Anim. Sci.* 7(1): 147-153

Adeyemo, G.O., and Longe, O.G. 2007. Effect of graded level of cotton seed cake on performance, Haematological and carcass characteristics of broilers fed from day to 8 weeks of age. *African Journal of Biotechnology*, 6, (8) 1064-1071.

Akinleye, S. B., Iyayi, E. A., and Afolabi. K. D. 2008. The Performance, Haematology and Carcass Traits of Broilers as Affected by Diets Supplemented with or without Biomin a Natural Growth Promoter. *World Journal of Agricultural Sciences* 4 (4): 467-470, 2008

Casewell, M., Friis. C., Marco. E., McMullin. P., and Philips. I. 2003. The European ban on Growth Promoting Antibiotics and Emerging consequences for Human and Animal Health. *Journal Antimicro. Chemo.* 52,159-161

Cheeke, P. R and Dierenfeld, E. S. 2010. Comparative animal nutrition and metabolism. First Edition. Cambridge University Press, Cambridge. Pg 133-134

Church, J. P., Judd, J.T., Young, C.W., Kelsag, J. L., Djum, W. W. 1984. Relationship among dietary constituent and specific serum clinical components of subjects eating self-selected diets. *Anim. J. Clinical. Nutrition*. 40: 1338-1340.

Clinical Diagnostics Division, 1990. Veterinary Reference Guide, Eastman Kodak Company, Rochester, New York.

Cooper. G. R., and McDaniel. V. 1970. Standard method in Serum Enzyme activity. *Clinical Chemistry* :6 :159

Dibner, J. J., and Richard, J. D. 2005: Antibiotics Growth Promoters in Agriculture, History and Mode of Action. *Journal of Poultry Science*, 84:634-643

Fairchild, A. S., Grimes, T.L, Jones F. J. Wineland M. J. Eden .F. W. Sefton A. E. 2001. Effects of Hen age, Bio-MOS and *Fl*avomycin. Poults susceptibility to oral *Escherichia coli* challenge. *Journal of Poultry Science*: 80, 562-571

Friedewald, W.T., Levy. R. I., Fredrickson. D. S. 1972. Estimation of the concentration of the LDL Cholesterol in plasma, without using Preparative ultracentrifuge. *Journal of Clinical Chemistry*.:18, :499-502.

Gowenlock, A. H., McMurray. J. R., MacLauchlan. D .M. 1988. Varley Practical Clinical Biochemistry 6th Edition CAC Publishers and Distributors. New Delhi. Pg: 477-549.

Hernandez, F., Garcia, V., Madrid, J., Orengo, J., Catala, P., Megais, M. D. 2006. Effect of Formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite level of Broiler chickens. *British Poultry Science*, 47:50-56.

Jain, N.C.1993. Schalms Veterinary Haematology 4th Edition, Philadelphia. USA. Publisher: Lea and Febiger. Chapter 4,63-70.

Kim, Y.Y, Kil, D. Y.,Oh, H. K and Han, I. K. 2005. Acidifiers as an alternative materials to Antibiotics in Animal Feed. Asian-Aust. *Journal of Animal Science*, 18, 1048-1060

Kirchgessner. M. and Roth. F. X. 1988. Fumaric acid as a feed additive in Pig nutrition. Pig News Information article, 3, 259-264.

Kohn. R.A., and Allen. M.S. 1995. Enrichment of Proteolytic activity relative to Nitrogen in Preparation from the rumen for in vitro studies. *Journal of Animal Feed Science and Technology*. 52.: 1-14.

Losonczy, S., Losonczy, M., Takacs, E. 1972. Formen und Erblichkeit der Praealbumine im Blutplasma und Eiinhalt von Henne. Prace i Materialy *Zootechnicze* (Warszawa) 15, 89-104.

Mitruka, B. M., and Rawnsley, H. M. 1977. Clinical Biochemical and Haematological Reference values in normal Experimental animals. New York. Masson Publishing. USA Inc. pg: 21-84.

Murwani, R., Bayuardhi, B. 2007. Broiler serum cholesterol and Glutamic oxaloactetic transaminase and their relation to antibiotics in feed and medication programmes in four Broiler producers in Semarang region-central Java Indonesia. *Int. J. Poultry. Sci.* 6:266-270.

Oluyemi, J. A. and Roberts, F. A. 2000. Poultry production in the warm wet climate. 1st Ed. Macmillan Press, Ltd, Nigeria

Onifade, A. A. and Odunsi, A. A. 1998. Efficacy of Procaine Penicillin as a growth promoter in broiler chicks fed low and high fibre diets in the Tropics. *Arch. Zootech*. 47:621-628

Onifade, A. A. 1993: Comparative utilization of three dietary fibre sources by broiler chickens. Ph.D. Thesis, University of Ibadan, Ibadan Nigeria.

Peters, T., Biamonte. G. T., and Doumas. B. T. 1982. Protein. (Total Protein), in Serum, Urine and Cerebrospinal *fl*uid, albumin in serum. Selected Methods of clinical Chemistry. vol: 9. W. R. Paulkner and S. Meites. Eds. Washington. D. C. America Association of Clinical Chemistry.

Partanen, K. H. 2001. Organic acids their Efficacy and modes of action in Pigs In: Gut Environment in Pigs. (A. Piva, K.E. Bach Knudsen and J.E Lindbergh, Eds) pp 201-218, University Press Nottingham, UK.

Sarinee, K.T., Boonrit, T., Vivat. C. 2008. Blood Haematological-Cholesterol Profile and Antibody Titer Response of Broilers with added Probiotic containing both Bacteria and Yeast or an Antibiotic in Drinking Water. *Thailand Journal of Veterinary Medicine* vol. 38 (4): 45-56.

SAS (1999), SAS User's Guide: SAS Inst., Inc, Cary, NC.

Schalm, O.W., Jain. N. C., and Carol. E. J. 1975. Veterinary Haematology. 3rd Edition. Publisher; Lea and Febiger Philadelphia.

Senkoylu. N., Samli. H. E., Kanter. M., Agma. A. 2007. In*fl*uence of a combination of formic and Propionic acids added to wheat and Barley based diets on the performance and gut Histomorphology of Broiler chickens. Acta *Veterianaria Hungaris*, vol, 55 (4) : 479-490

Sheikh, A., Tufail, B., Gulam, A. B, Masood, S. M., Manzoor, R. 2006. Effect of Dietary supplementation of Organic acids on Performance, Intestinal histomorphology and Biochemistry of Broiler chickens.

Steiner, T. 2006. The potential benefits of Natural Growth Promoters. *Journal of Feed Technology,*10 (2),26-28

Whitehead, C. C and Griffin, H. D. White head, C. C., and Griffin, H. D. 1982. Plasma lipoprotein concentration as an indicator offatness in broilers: effect of age and diet. *British Poultry Science*, 23: 299-305.

Wary. C, and Davis, R. H 2000. Competitive Exclusion an-alternative to Antibiotics. *Journal of Veterinary Medicine*. I; 159, :107-108