RESPONSE OF BROILER CHICKS TO VIRGINIAMYCIN AND DIETARY PROTEIN CONCENTRATIONS IN THE HUMID TROPICS

RESPUESTA DE BROILERS A LA VIRGINIAMICINA Y A LAS CONCENTRACIONES PROTEICAS DE LA DIETA EN EL TRÓPICO HÚMEDO

Odunsi, A.A.[1], A.A. Onifade[2] and G.M. Babatunde[3]

[1]Department of Animal Prodiction and Health, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

[2]Department of Botany and Microbiology, Faculty of Science, Kuwait University, P.O. Box 5969, Safat 1060, Kuwait.

[3]Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

ADDITIONAL KEYWORDS

Feed additive. Poultry. Growth. Heamatology.

PALABRAS CLAVE ADICIONALES

Aditivos alimenticios. Avicultura. Crecimiento. Hematología.

SUMMARY

The performance, protein utilization, haematology and serum chemistry of broiler chicks fed 180, 210 and 240 g/kg protein levels without (0 ppm) or with (20 ppm) Virginiamycin (Vm) as a growth promoter during the starting phase was investigated.

Body weight, feed intake and feed conversion efficiency increased ($p<0.05$) with protein concentration. Additionally, Vm significantly ($p<0.05$) increased daily gain and feed efficiency above the unsupplemented groups. Protein intake and excretion correlated ($r= 0.993$) positively, however, groups on supplements had reduced excreta protein content thus culminating into higher daily (absolute) and apparent (relative) protein retention than groups without. The haematological and serum biochemical values in the broilers generally suggest adequate and healthy nutrition. A compendium of the results indicate the differential effects of dietary protein levels on the response of broiler chicks to Vm fed as a growth promoter.

RESUMEN

En broilers alimentados con raciones que contenían 180, 210 y 240 g/kg de proteína, sin (0 ppm) o con (20 ppm) virginiamicina (Vm) como promotor del crecimiento, se investigó, durante la fase de inicio, la eficacia, utilización de la proteína, hematología y química del suero.

Al aumentar la concentración proteica, lo hicieron ($p<0,05$) el peso corporal, ingestión de alimento y eficacia de conversión del pienso. Además, la Vm incrementó significativamente ($p<0,05$) la ganancia diaria de peso y la eficacia

[1]Corresponding author.

[2]The use of Virginiamycin is forbidden in the EU and other countries, but many others where its use is not restricted can obtain considerable profit of including antibiotics as diet ingredients.

de conversión del alimento respecto a los grupos no suplementados. La ingestión y excrección de proteína estuvieron positivamente correlacionadas (r= 0,993). Sin embargo, los grupos que recibieron el suplemento mostraron una reducción del contenido proteico de la excreta determinando una superior retención proteica diaria (absoluta) y aparente (relativa) que la de los que no lo recibieron. Los valores hematológicos y bioquímicos del suero de los broilers sugieren en general una nutrición suficiente y saludable. Como compendio los resultados indican efectos diferenciales de los niveles de proteína del pienso sobre la respuesta de los broilers a la Vm cuando se emplea como promotor del crecimiento.

INTRODUCTION

Virginiamycin is an effective antibiotic against gram positive microorganisms and like some other antibiotics enhances the performance of broiler chickens (Bunyan *et al.*, 1977; Miles *et al.*, 1984a, Harms *et al.*, 1986). However, broiler chicks may exhibit differential responses depending on diet composition, nutrient levels, age, stocking density and genetic make-up (Dafwang *et al.*, 1987; Bartov, 1992a, b; Onifade and Babatunde, 1997; Odunsi and Onifade, 1998). Conflicting information are available on the effects of these largely exogenous determinants of response of chickens fed different growth promoters. For instance, Bartov (1992a) concluded that the response of broiler chickens to virginiamycin is not dependent on energy: protein ratio or energy concentration whereas Bartov (1992b) affirmed that the response of broiler chicks to avoparcin increased with dietary energy concentration. Onifade and Babatunde (1997) concluded that growth of chicks fed a high fibre diet was improved by antibiotic supplementation whereas Odunsi and Onifade (1998) reported the absence of synergistic growth promotion by supplemental oil and zinc bacitracin in broiler chicks. Foster and Stevenson (1983) showed that the response of broiler chicks to avoparcin and zinc bacitracin was not dependent on dietary protein concentration. Earlier, March *et al.* (1978) observed that the response of chickens to virginiamycin incorporated in diets varying in their grain source, fat and protein concentrations were essentially the same, while Hedde (1983) later reported that the effect of virginiamycin on improving broiler performance was more pronounced when diets of low energy concentration were used.

This controversy provides a testable hypothesis, hence we evaluated the growth performance, protein utilization, haematology and serum biochemistry of broiler chickens fed three protein concentrations each with or without supplemental virginiamycin in a humid tropical environment.

MATERIALS AND METHODS

A 3x2 factorial experiment comprising three dietary protein concentrations: 180, 210 and 240 g/kg and two concentrations: 0 and 20 ppm of virginiamycin (Vm) was designed. The resultant six diets **(table I)** were fed to 1 day old mixed sex broiler chicks of Hubbard strain up to 35 days of age. Each diet was fed to forty broiler chicks randomly divided into four equal replicates. The broiler chicks were

reared in electrically heated floor pens up to 28 days of age after which brooding was discontinued. Broiler chicks were offered feed and water *ad libitum* throughout the experimental period. Individual body weights were determined weekly, while feed intakes were computed weekly on replicate basis. Between 28 and 35 days of the experiment, data on protein utilization were collected. Eight 28 day old broilers of mean weight per treatment (two per replicate) were randomly selected and kept individually in metabolism cages for protein balance studies. Feed allocation to the birds was uniformly maintained at 75 g/broiler/day. After three days of adjustment, total collection of excreta was carried out. Daily excreta collections were dried in a forced-draft oven at 60°C for 48 hours and later analysed for N content. Blood specimens were terminally collected in the 35th day from 8 overnight fasted broiler chicks per treatment through the jugular vein using sterile syringes and needles. Haematological samples were collected in EDTA-treated bottles; serum chemistry samples were collected without the anticoagulant.

Packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and haemoglobin (Hb) concentrations were determined using Wintrobe's microhaematocrit, improved Neubauer haemocytometer and methaemoglobin methods respectively. The erythrocytic mean indices: corpuscular volume (MCV); corpuscular haemoglobin (MCH) and corpuscular haemoglobin concentration (MCHC) were computed according to Jain (1986). Serum total protein, albumin, globulin and urea were analysed using Sigma assay kits; creatinine (Scott, 1965) and uric acid (Henry *et al.*, 1957). Diets and excreta were analysed for N according to AOAC (1990).

Data collected were subjected to analysis of variance of factorial experiment (Steel and Torrie, 1980), and differences between means were separated according to Duncan (1955).

Table I. *Composition of the basal diets (g/kg).* (Composición de las dietas basales (g/kg)).

Crude protein	180	210	240
Maize	680.0	583.0	486.5
Groundnut cake	243.0	340.0	436.0
Fish meal	30.0	30.0	30.0
Bone meal	25.0	25.0	25.0
Oyster shell	15.0	15.0	15.0
Salt	2.0	2.0	2.0
Premix[1]	2.5	2.5	2.5
Methionine	2.5	2.5	2.5
Virginiamycin*	+/-	+/-	+/-
Chemical analysis			
Crude protein g/kg	180.5	210.4	240.1
Metabolizable energy[2]	12.55	12.20	12.05

*The basal diets were supplemented without (-) or with (+) 20 ppm Virginiamycin.

[1]Premix supplied the following vitamins and minerals per kg of diet: A, 10000 i.u; D, 3000 i.u; E, 8.0 i.u; K, 2.0 mg; B_1, 2.0 mg, B_6, 1.2 mg; B_{12}, 0.12 mg; niacin, 1.0 mg; panthotenic acid, 7.0 mg; folic acid, 0.6 mg; choline chloride; 500 mg; Fe, 60 mg; Mn, 80 mg; Mg, 100 mg; Cu, 8.0 mg; Zn, 60 mg; Co 0.45 mg; 1,2.0 mg and Se, 0.1 mg.

[2]Calculated (MJ/Kg).

RESULTS

Table II summarises the effects of dietary protein concentration and

supplemental Vm on the growth of broiler chicks. Body weights of broiler chicks at 35 days of age and average daily gain on the unsupplemented diets increased significantly ($p<0.05$) and correlated positively ($r= 0.98$) with the unsupplemented diets. Peformance enhancement sequel to supplemental Vm correlated ($r= 0.99$) with dietary protein concentration. Feed consumption of broilers increased ($p<0.05$) correspondingly ($r= 0.996$) with protein concentration. Broiler chicks fed supplemented diets exhibited a comparable ($p>0.05$) quantitative feed consumption pattern with their unsupplemented counterparts, but for the significantly ($p<0.05$) augmented intake on 240 g/kg dietary protein. Amongst the unsupplemented groups, broilers fed 210g/kg achieved the best ($p<0.05$) feed: gain ratio while the lowest ($p<0.05$) was recorded for those fed 180 g/kg dietary protein. Vm stimulated significantly ($p<0.05$) better feed conversion efficiency but almost ($p= 0.071$) increasingly with the protein concentrations.

Table II. *Performance and protein utilization indices by broiler chickens fed three dietary protein concentrations with or without supplemental virginiamycin.* (Eficacia e índices de utilización de la proteína por broilers alimentados a tres niveles proteicos y con o sin virginiamicina suplementaria).

Crude Protein g/kg	180		210		240		
Virginiamycin (ppm)	-	20	-	20	-	20	SEM
Final live weight g	650.0^{a}	686.5^{b}	800.0^{c}	859.6^{d}	870.0^{d}	975.0^{e}	22.12
Weight gain g	610.0^{a}	646.5^{b}	760.0^{c}	819.6^{d}	830.0^{d}	935.0^{e}	21.60
Daily weight gain g	17.42^{a}	18.47^{b}	21.71^{c}	23.42^{d}	23.71^{d}	26.71^{e}	0.43
Feed intake g/kg	43.5^{a}	44.73^{a}	51.50^{b}	54.2^{b}	57.5^{c}	62.0^{d}	1.21
Feed/gain	2.50^{a}	2.42^{b}	2.37^{c}	2.31^{c}	2.44^{b}	2.32^{c}	0.01
Protein intake g/day	13.50^{a}	13.50^{a}	15.75^{b}	15.75^{b}	18.00^{c}	18.00^{c}	0.34
Protein output g/day	4.93^{b}	4.60^{a}	5.86^{c}	5.30^{b,c}	6.46^{d}	5.80^{c}	0.09
Protein retained g/day	8.57^{a}	8.90^{a}	9.89^{b}	10.45^{b}	11.54^{b,c}	12.2^{c,d}	0.31
Apparent protein retention percent[1]	63.48^{a}	65.93^{b}	62.79^{a}	66.35^{b}	64.11^{a}	67.78^{b,c}	0.19
Protein intake efficiency[2]	1.29^{a}	1.37^{b}	1.38^{b}	1.49^{c}	1.32^{a}	1.48^{c}	0.02
Protein retention efficiency[3]	2.03^{a}	2.08^{a}	2.20^{a,b}	2.24^{a,b}	2.06^{a}	2.19^{a}	0.01

Data are means of four samples for each parameter analysed.
a,b,c,d: Means in the same row without a common superscript are significantly ($p< 0.05$) different.
[1]Apparent protein retention= (protein retained - protein intake) x 100.
[2]Protein intake efficiency= Daily gain - protein intake.
[3]Protein retention efficiency= Daily gain - protein retained.

Table III. *Haematological and serum biochemical values in broiler chickens fed three dietary protein concentrations with or without supplemental Virginiamycin.* (Valores hematológicos y bioquímicos del suero en broilers alimentados con tres niveles proteicos con y sin suplemento de virginiamicina).

Crude protein g/kg	180		210		240		
Virginiamycin ppm	-	20	-	20	-	20	SEM
Packed cell volume*	31.5^{a}	32.2^{a}	33.0^{a}	34.4^{a,b}	35.5^{a,b}	36.7^{b,c}	1.21
Haemoglobin p.100	10.4^{a}	10.9^{a}	11.0^{a}	11.5^{a,b}	11.9^{a,b}	12.2^{b,c}	0.09
Red blood cell 10^{6}/m	2.9^{a}	2.8^{a}	2.9^{a}	2.9^{a}	3.1^{a,b}	3.2^{a,b}	0.02
Mean Corpuscular							
- volume Fl	116.7^{a,b}	115.0^{a}	113.8^{a}	118.6^{a,b}	114.5^{a}	114.7^{a}	1.50
- heamoglobin Pg	38.5	38.9	37.9	39.7	38.4	38.1	0.63
- haemoglobin con-							
centration*	33.0	33.9	33.3	33.4	33.5	33.2	0.45
Total Protein g/l	40.1^{a}	41.5^{a}	45.0^{b}	46.4^{b}	54.0^{b,c}	60.0^{d}	2.50
Albumin g/l	13.2^{a}	13.5^{a}	15.0^{b}	15.2^{b}	15.8^{b}	16.5^{c}	0.14
Globulin g/l	26.9^{a}	28.0^{a,b}	30.0^{b}	31.2^{b}	38.2^{c}	43.5^{d}	1.12
Creatinine mg/l	9.0	6.8	6.9	6.5	6.6	6.4	0.08
Uric acid mg/l	15.2^{a}	16.5^{a,b}	17.9^{b}	19.6^{b,c}	21.3^{c}	23.8^{c,d}	1.1
Urea mg/l	181.1	175.4	150.9	140.0	132.6	120.8	5.4

^{a,b,c}Means in the same row without a common superscript are significantly ($p<0.05$) different.
Data are means of eight samples for each parameter analysed.
*p.100

The effects of protein concentration and supplemental Vm on the protein utilization **(table II)** showed that daily protein intakes were similar on each protein concentration because of the equalized feed allocation; it however increased ($p<0.05$) correspondingly with the dietary protein level. Broilers excreta nitrogen content increased ($p<0.05$) along the protein concentration; however the antibiotic significantly ($p<0.05$) reduced quantitative nitrogen excretion of broilers. The amount of protein retained daily by the chicks also increased ($p<0.05$) with the dietary protein, and was numerically ($p>0.05$) higher on supplemented diets containing the same protein content.

Apparent protein retention of broilers exhibited a congruent pattern as for daily protein retention, but for the significantly ($p<0.05$) higher values on Vm supplemented diets. Broiler fed supplemental Vm progressively ($p<0.05$) enhanced efficiency of utilization of protein consumed above the unsupplemented groups. The groups fed unsupplemented diets showed no particular trend save the high ($p<0.05$) protein efficiency on 210 g/kg protein. Protein retention efficiency calculated as a ratio of daily gain: protein retained

per day was similar amongst the broiler chicks fed different protein concentrations; though groups fed 210 g/kg protein tended to be more ($p<0.05$) optimally efficient.

Table III displays the effects of dietary protein concentration and supplemented Vm on the haematological and serum biochemical values in broiler chicks. The haematological parameters: PCV, RBC; Hb, WBC values in the broilers increased numerically with protein concentration, and were slightly higher ($p>0.05$) on supplemented diets. The absolute indices: MCV; MCH and MCHC were essentially comparable ($p>0.05$) on the treatments. Serum biochemical values revealed that total protein, albumin, and globulin concentrations in the blood of the broilers responded incrementally ($p<0.05$) to the protein concentration, while Vm induced no significant ($p>0.05$) difference on similar concentration except on 240 g/kg protein diet. Serum urea concentration was non-significantly ($p>0.05$) and inversely ($r= 0.997$) related with protein concentration, while uricacid concentration varied directly ($r= 0.996$) with protein concentration albeit insignificantly ($p>0.05$). Creatinine varied insignificantly ($p>0.05$) in the serum of broilers fed the experimental diets.

DISCUSSION

Broiler chicks attained progressive growth rates on increasing dietary protein concentrations in agreement with the findings of Babatunde and Fetuga (1976); Olomu and Offiong (1980), Deschepper and Degroote (1995), Odunsi *et al.* (1997). Vm supplementation further remarkably enhanced 35 day body weight and average daily gain of broilers irrespective of the protein concentration. This vindicates growth promoting properties of Vm as earlier documented (Bunyan *et al.*, 1977; Miles *et al.*, 1984a,b, Belay and Teeter, 1996). Finding performance enhancement on the supplemented groups to be proportionate ($r= 0.993$) with protein concentration resembled the effects of b-adrenergic agonists on growth and muscle accretion (Mitchell *et al.*, 1994: Oksbjerg *et al.*, 1984). Bartov (1992b) similarly observed higher growth rate of broilers fed avoparcin on high than low energetic concentrations. The present result contrasted with those of March *et al.*, (1978), Foster and Stevenson (1983) and Bartov (1992a) all of who reported lack of diet composition, protein and energy concentrations on the response of broilers to different antibiotics. Elucidating further, the parallel growth improvement with protein concentration without ($r= 0.983$) and with ($r= 0.996$) Vm ostensibly indicates that broilers are being increasingly satisfied. Satisfactory growth rates were earlier obtained betwen 23 and 26 percent crude protein in the tropics (Babatunde and Fetuga, 1976; Olomu and Offiong, 1980; Odunsi *et al.*, 1997), and 23 percent in the temperate (NRC, 1994).

The highly positive ($r= 0.996$) correlation between feed intakes and dietary protein concentration **(table II)** corresponded with the reports of Odunsi *et al.*, (1997) but at variance with the findings of Oyeleke *et al.* (1987) and Deschepper and Degroote

(1995). Supplemental Vm noticeably stimulated 2.83, 5.24 and 7.83 percent increases in feed intake and this resulted into 4.17, 7.92 and 12.65 percent growth increments of broilers fed 180, 210 and 240 g/kg protein concentrations respectively. Understandably, the greater feed intakes on supplemented diets provided additional nutritive substrates for enzymatic transformation which explicably translated into superior body weights. The growth increment sequel to Vm however, were greater than the magnitude of escalation in feed intake. This observation deductively infers that: (I) growth rate of the broiler chicks is more sensitive than feed intake to Vm supplementation and (II) there is interaction or differential responses of broilers to Vm and protein concentrations.

Although feed conversion efficiency of broiler chicks fed unsupplemented and supplemented diets respectively correlated moderately (r = 0.461) and highly (r= 0.822) with protein concentration; the parameter seems to be less sensitive than growth rate to antibiotic supplementation. Reasons could not be readily adduced for this observation. Perhaps, Vm mechanistically reduced and/or eliminated intestinal growth retarding microorganisms without enhancing nutrient uptake at the absorptive surfaces in the broiler chicks. This hypothesis derives support from the reported antimicrobial efficacy and stability of streptomycin in chicken intestine (Jefferies *et al*., 1977; Bunyan *et al.*, 1977); and the congruous observation by Wagner and Thomas (1978) that significant growth improvement followed procaine penicillin elimination of intestinal growth retarding organisms in broiler chicks fed rye.

In **table II**, protein intake and excretion correlated (r= 0.993) positively, and agreeably with findings of Deschepper and Degroote (1995). It was also observed that supplemental Vm beneficially reduced excreta protein content up to 11.38 percent in broilers fed 240 g/kg protein. This may be consequent upon the reduction in bacterial contributions to excreta nitrogen/protein. Parsons *et al*. (1982) estimated bacterial contribution to excreta to be more than 25 percent. It may also partly reflect true absorption of the dietary protein considering the superior apparent protein retention, feed and protein eficiencies and the weight gain on the supplemented diets.

The haematological values in the broilers are analogous with normal ranges (Meluzzi *et al.*, 1992) and corroboratively (Odunsi *et al.*, 1997; Odunsi and Onifade, 1998) suggest adequate and healthy nutrition. However, the preponderant values in the antibiotic supplemented groups justifies the established positive relationship between haematological parameters and performance of animals (Babatunde and Pond, 1987). The higher serum total protein in broilers fed 240 g/kg protein indicates superior protein nutrition and adequacy, while the inverse relationship (r= 0.997) of urea concentration with dietary protein is consistent with reported trends (Babatunde and Pond, 1987). Finding a direct relationship between uric acid and protein concentration agrees with (Hevia and Clifford, 1977; Belay and Teeter, 1996) while the apparent similarity in creatinine concentration is indicative of normal

muscle metabolism (Latner, 1975).

The findings indicate the differential effects of dietary protein concentration in the response of broiler chicks to supplemental Vm as a feed additive in a warm humid tropical environment.

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