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Responses of Broilers Fed Guineacorn/Palm Kernel Meal Based Ration to Supplemental Biotin

Raheem A Oloyo

Department of Science Laboratory Technology, Federal Polytechnic, PMB 50 Ilaro, Ogun State, Nigeria

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ABSTRACT

Seven duplicate floor pens with 20 day-old commercial broiler chicks each were fed a practical type broiler diet formulated with guineacorn/palm kernel meal and supplemented with graded levels of biotin (0·00, 0·04, 0·08, 0·12, 0·16, 0·20 and 0·24 mg kg^{-1} feed) for a period of 6 weeks. Significantly poorer feed utilisation and carcass characteristics, higher blood lipid, lower blood glucose, lower pyruvate carboxylase activity in the liver, higher liver and kidney weights (% live weight), more lipid deposition in these organs coupled with more frequent incidences of foot dermatitis, higher mortality due to fatty liver and kidney syndrome (FLKS) and abnormal development of leg bone in broilers maintained on the diet without biotin supplementation indicated that they suffered vitamin deficiency. However, the responses of experimental birds indicated that a dietary supplement of 0·20 mg biotin kg^{-1} feed was adequate for the promotion of good performance and prevention of dermal lesions, FLKS mortality and leg deformities.

Key words: Biotin, broiler chicken, guineacorn, palm kernel meal.

INTRODUCTION

Wide variation in the values reported in the literature as to the biotin requirement of broilers has been attributed to differences in the relative bioavailability of the vitamin in feed ingredients used in the test diets. In Nigeria, the types of feed ingredient used in broiler diet formulations vary with the geographical regions. For example, whereas maize is the energy source in broiler rations in the southern region, guineacorn is the most common energy source in the northern part.

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However, groundnut cake is the major protein source in use throughout the country. Consequently, Ogunmodede (1978) established the biotin requirement of broilers fed diets based on either maize/groundnut cake ($0{\cdot}12\ \mathrm{mg\ kg^{-1}}$) or guineacorn/groundnut cake ($0{\cdot}15\ \mathrm{mg\ kg^{-1}}$) and attributed the difference in requirement values to reduced bioavailability of biotin in guineacorn.

Consequent upon the high cost and inadequate supply of groundnut cake in recent times, research attention has been focused on the search for an alternative vegetable protein source in broiler diets. Although the use of palm kernel meal as a substitute for groundnut cake in broiler diets in Nigeria is becoming increasingly popular, the problem of biotin requirement of broilers fed diets based on guineacorn/palm kernel meal still remains unsolved. More especially it has been suggested that dietary fibre might be interfering with the absorption of free biotin from the bird's gut with consequent reduction in bioavailability of the vitamin from cereal grains such as corn, guineacorn, wheat and triticale (Misir and Blair 1984). Since palm kernel meal is characterised by its high fibre content, it is necessary to estimate the supplemental level of biotin required by broilers when fed palm kernel meal based rations.

The study reported here therefore aimed to estimate the amount of supplemental biotin required by broilers fed a guineacorn/palm kernel meal based diet.

MATERIALS AND METHODS

Birds, diets and husbandry

A total of 280 day-old commercial broilers were randomly alloted to seven supplemental biotin groups (0·00, 0·04, 0·08, 0·12, 0·16, 0·20 and 0·24 mg biotin $\mathrm{kg^{-1}}$ diet) and there were two floor pens per treatment group with 20 chicks per pen. The basal experimental diet was obtained by formulating a practical diet containing guineacorn and palm kernel meal (Table 1) which was then supplemented with feed-grade biotin (Rovimix H-2, Roche, Switzerland) such that seven graded levels of the vitamin were obtained. Biotin content of the basal diet was assayed microbiologically prior to vitamin supplementation. Chicks were raised in 14 floor pens, each of $4{\cdot}2\ \mathrm{m^2}$ floor area and containing dry wood shavings litter, two 4-litre plastic drinkers, a trough feeder and a 100-W tungsten filament lamp. Experimental birds were maintained on the respective dietary treatments for a period of 6 weeks during which they had free access to feed and water at all times, and routine vaccinations were administered. All birds that died were sent to the Veterinary Division of the State Ministry of Agriculture and Natural Resources, Ilaro, for post-mortem examination.

Weekly records were kept of: feed intake; weight gain; feed efficiency (gain/feed intake); incidence of dermatitis (percentage of birds within a treatment group showing mild to very severe signs); mortality due to fatty liver and kidney syndrome, FLKS (percentage of death due to FLKS within a treatment group); and incidence of leg deformities in terms of the percentage of birds within a

TABLE 1
Composition of basal experimental diet

	%
Guineacorn	55·6
Palm kernel meal	18·0
Blood meal	8·9
Fish meal	2·2
Brewer's grain	11·0
Oyster shell	1·0
Bone meal	2·0
Vitamin/mineral premix[a]	0·1
Salt (NaCl)	0·2
Palm kernel oil	1·0
Total	100·0
Analysis	
Biotin (mg kg^{-1})	0·04
Calculated	
Crude protein (%)	21·13
Metabolisable energy (kcal kg^{-1})	2716·33
ME/CP	128·55
Fat (%)	3·50
Linoleic acid (%)	1·30

[a] Vitamin/mineral premix supplied the following vitamins and mineral elements per kg of feed: vitamin A, 1200 IU, vitamin D, 2500 IU, vitamin E, 10 IU, menadione sodium bisulphite (vitamin K) 1·5 mg, vitamin B_1, 2·5 mg, vitamin B_2, 5 mg, cholin chloride, 500 mg, calcium D-pantothenate, 10 mg, nicotinic acid, 35 mg, vitamin B_6, 4 mg, vitamin B_{12}, 0·02 mg, iron, 50 mg, manganese 150 mg, copper, 2·5 mg, zinc, 45 mg, cobalt, 0·2 mg, selenium, 0·08 mg, iodine, 1·4 mg.

treatment group showing (i) legs with crooked toes, (ii) legs with bowed or twisted toes, or (iii) difficulty in standing or walking.

At the fourth and sixth weeks, 2 ml of blood was collected in a heparinised container from wing veins of each of four replicate samples of experimental chicks randomly selected from each treatment group for the determination of glucose, total lipid and free fatty acid contents. Selected samples were then slaughtered, and the livers and kidneys were excised, drained of fluid with blotting paper and weighed. These organs were also kept for subsequent estimation of total lipid and triglyceride contents. Pyruvate carboxylase activity in livers was also measured.

Bird samples selected at the sixth week of feeding trial were dressed for carcass characteristic evaluation. The carcasses were weighed and dressing percentage was calculated. Bones in the carcass were carefully removed and the edible meat was separated. Care was taken to prevent loss of meat to the bones. Every effort was made to dress all the carcasses as identically as possible. Total edible meat and total bone from each of the carcasses were weighed and the meat to bone ratio was calculated. Meat and bone were also expressed as percentages of carcass weights.

Analytical procedure

(1) Blood samples were deproteinised by the addition of barium hydroxide and zinc sulphate solutions prior to the estimation of glucose (Dubois *et al* 1956).
(2) Total lipid content was determined by the method of Folch *et al* (1957).
(3) Triglyceride content was estimated as described by Fletcher (1968).
(4) Free fatty acid content was determined by the method of Pearson (1976).
(5) Pyruvate carboxylase activity in liver was measured in accordance with the procedure of Utter and Keech (1963).
(6) Biotin content of the basal diet was assayed by the method of Wright and Skeggs (1944).

Statistical analysis

The results obtained in the study were subjected to analysis of variance in accordance with the procedures of Steel and Torrie (1960). Significantly different treatment means were separated by the multiple range test of Duncan (1955).

RESULTS AND DISCUSSION

In order to estimate supplemental dietary biotin required by broilers fed a guineacorn/palm kernel meal based ration, birds were given the basal diet (Table 1) supplemented with graded levels of the vitamin. The lowest supplemental level of 0·04 mg kg^{-1} feed was about a half, a third and a quarter of the values estimated as requirement by Wagstaff *et al* (1961), Ogunmodede (1978) and Whitehead and Bannister (1978) respectively. The highest supplemental level of 0·24 mg kg^{-1} feed was six times the lowest. Thus a wide range of dietary biotin level was tested.

Performance of experimental broilers is shown in Table 2. Whereas feed intake and body weight gain were significantly affected by dietary supplementation of biotin, feed efficiency was not. Birds given 0·00–0·16 mg biotin kg^{-1} feed consumed significantly less feed and gained less weight than those given 0·20 and 0·24 mg kg^{-1} feed. Poor feed intake observed in birds given 0·00–0·16 mg kg^{-1} feed might be due to reduction in free movement of the birds as a result of the abnormal development of leg bones as well as the dermal lesions developed in the feet. The result therefore tends to suggest that a supplemental biotin level of 0·16 mg kg^{-1} feed was not adequate for efficient feed utilisation.

Biotin-related features in experimental broilers are shown in Table 2. Foot dermatitis characteristic of biotin deficiency (Patrick *et al* 1942; Ogunmodede 1978) developed as early as the first week of feeding in birds given no supplemental biotin and as late as the fifth week in those given 0·16 mg biotin kg^{-1} feed. However, no incidence was observed in those given 0·20 and 0·24 mg biotin kg^{-1} feed throughout the period of experimentation. In affected birds, foot pads were

TABLE 2
Performance of experimental broilers at 42 days

Parameter	*Supplemental biotin (mg kg^{-1} feed)*							
	0	*0·04*	*0·08*	*0·12*	*0·16*	*0·20*	*0·24*	*±SEM*
Biotin-related features								
Incidence of dermatitis (%)	65·0^{a}	70·0^{a}	50·0^{ab}	10·0^{bc}	2·5^{c}	0·0^{c}	0·0^{c}	11·21
FLKS mortality (%)	15·0^{a}	10·0^{ab}	10·0^{ab}	7·5^{b}	5·0^{bc}	0·0^{c}	0·0^{c}	1·94
Incidence of leg deformities (%)	12·5^{a}	12·5^{a}	7·5^{ab}	5·0^{bc}	5·0^{bc}	0·0^{c}	0·0^{c}	1·81
Feed utilisation								
Daily feed intake per bird (g)	80·58^{b}	84·86^{b}	85·58^{b}	92·00^{b}	93·72^{b}	109·86^{a}	109·28^{a}	4·10
Daily weight gain per bird (g)	20·96^{b}	22·92^{b}	22·26^{b}	25·76^{b}	25·30^{b}	32·90^{a}	31·60^{a}	1·62
Feed efficiency	0·26	0·27	0·26	0·28	0·27	0·30	0·29	0·005
Carcass characteristics								
Carcass weight (g)	518·4^{b}	537·2^{b}	561·0^{b}	581·0^{b}	689·0^{a}	706·8^{a}	687·4^{a}	28·04
Dressing percentage	60·3^{b}	61·1^{b}	62·6^{b}	63·5^{b}	68·8^{a}	69·0^{a}	68·9^{a}	1·36
Total edible meat (g)	336·6^{b}	346·8^{b}	363·4^{b}	375·8^{b}	447·2^{a}	471·2^{a}	454·4^{a}	19·71
Meat (% carcass weight)	64·9^{b}	64·6^{b}	64·8^{b}	64·7^{b}	64·9^{b}	66·7^{a}	66·1^{a}	0·29
Total bone (g)	182·0^{b}	190·6^{b}	197·6^{b}	205·4^{b}	241·8^{a}	235·6^{a}	233·0^{a}	8·44
Bone (% carcass weight)	35·1^{a}	35·4^{a}	35·2^{a}	35·3^{a}	35·1^{a}	33·3^{b}	33·9^{b}	0·29
Meat:bone ratio	1·85^{b}	1·82^{b}	1·84^{b}	1·83^{b}	1·85^{b}	2·00^{a}	1·95^{a}	0·02

$^{a-c}$ Within a row values followed by different superscript letters are significantly different at $P<0·05$.

swollen and contained haemorrhagic fissures. Cracked and swollen areas occurred on pads on the bottom of the feet. Whereas Ogunmodede (1978) reported that 0·12 mg biotin per kg feed prevented dermal lesions in broiler chicks, Whitehead and Bannister (1978) reported that the severity of foot lesions was only reduced by increasing the supplemental biotin level but the lesions were not eliminated at the highest level studied (0·50 mg kg^{-1} feed). The results obtained in this trial showed that 0·20 mg biotin per kg feed was required for prevention of dermal lesions in experimental broilers.

Mortality due to fatty liver and kidney syndrome (FLKS), a biotin deficiency symptom in chicks (Payne *et al* 1974), was recorded in this trial among chicks given 0·00–0·16 mg kg^{-1} supplemental biotin per kg feed (Table 2). FLKS, a metabolic disorder, is characterised by morbidity followed by death in young chicks, usually in the age range of 3–5 weeks, but it can occur as early as 10 days and as late as 56 days of age and its incidence can be eliminated by dietary biotin supplementation (Bannister 1976; Whitehead *et al* 1976). In this study, FLKS mortality was recorded as early as the first week of feeding in birds given 0·04 mg supplemental biotin per kg feed, and as late as the fifth week in those given diet without biotin supplementation. Percentage FLKS mortality recorded throughout the experimentation period showed that 0·20 mg biotin per kg feed prevented the occurrence of the syndrome.

Histological examination and chemical analysis of the liver and kidney of FLKS-affected chicks showed that these organs were enlarged and markedly involved in fatty infiltration (Whitehead *et al* 1973; Whitehead 1975; Wight and Siller 1975). Higher liver and kidney weight as a proportion of live weight coupled with higher lipid contents of these organs in birds given 0·00–0·12 mg biotin per kg feed (Tables 3 and 4) reflected lipid deposition in the two organs due to biotin deficiency, hence the higher incidence of FLKS mortality. Triglyceride concentration and triglyceride as a proportion of total lipid in the two organs were also higher in birds given 0·00–0·12 mg biotin per kg feed (Tables 3 and 4). This result is in agreement with those reported by Johnson *et al* (1972) and Whitehead (1975) who noted that the extra lipid in liver and kidneys of FLKS-affected chicks was mainly triglyceride.

In FLKS-affected chicks, Whitehead *et al* (1973), Bannister *et al* (1975) and Balnave *et al* (1977) observed an elevated level of plasma free fatty acid and a markedly reduced glucose level. Whitehead *et al* (1976) also indicated that the primary abnormality in the FLKS condition is the failure of hepatic gluconeogenesis via pyruvate carboxylase, a biotin-dependent enzyme. This results in severe hypoglycaemia which is believed to be the cause of death. In this trial, broiler chicks given 0·00–0·12 mg biotin per kg feed had significantly lower liver pyruvate carboxylase activity and lower blood glucose concentration but had higher blood total lipid and free fatty acid concentrations (Tables 5 and 6). This result indicated a hypoglycaemic condition, hence the higher FLKS mortality recorded in these groups of birds. In effect, liver, kidney and blood lipid, and blood glucose values as well as pyruvate carboxylase activity in liver, suggested that a supplemental biotin level of 0·16 mg kg^{-1} feed given to birds was barely adequate.

TABLE 3
Weight and lipid content of liver

Parameter	*Age (weeks)*	*Supplemental biotin (mg kg^{-1} feed)*							
		0	*0·04*	*0·08*	*0·12*	*0·16*	*0·20*	*0·24*	*±SEM*
Weight (g)	4	20·2^{a}	20·6^{a}	20·4^{a}	19·6^{ab}	18·8^{bc}	18·2^{c}	18·6^{c}	0·34
	6	31·2	30·0	29·8	30·2	30·8	31·6	30·6	0·23
Weight (% live weight)	4	3·56^{a}	3·42^{a}	3·48^{a}	3·35^{a}	2·70^{b}	2·50^{b}	2·62^{b}	0·16
	6	3·62	3·41	3·33	3·30	3·08	3·08	3·06	0·07
Total lipid (mg g^{-1})	4	302·1^{a}	288·2^{a}	260·5^{a}	243·7^{a}	158·3^{b}	164·2^{b}	161·9^{b}	21·98
	6	175·3	169·8	171·6	164·9	166·6	168·8	172·1	1·23
Triglyceride (mg g^{-1})	4	206·0^{a}	199·1^{a}	170·1^{a}	158·4^{a}	73·3^{b}	75·2^{b}	75·6^{b}	21·10
	6	84·8	80·3	79·1	79·3	79·6	79·2	81·2	0·71
Triglyceride (% total lipid)	4	68·2^{a}	69·1^{a}	65·3^{a}	65·0^{a}	46·3^{b}	45·8^{b}	46·7^{b}	3·89
	6	48·4	47·3	46·1	48·1	47·8	46·9	47·2	0·27

$^{a-c}$ Within a row values followed by different superscript letters are significantly different at $P < 0·05$.

TABLE 4
Weight and lipid content of kidneys

Parameter	*Age (weeks)*	*Supplemental biotin (mg kg^{-1} feed)*							
		0	*0·04*	*0·08*	*0·12*	*0·16*	*0·20*	*0·24*	*±SEM*
Weight (g)	4	7·4	7·2	7·4	7·2	7·2	7·8	7·0	0·09
	6	10·4	11·2	10·8	11·2	11·6	12·6	11·4	0·24
Weight (% live weight)	4	1·32^a	1·20^a	1·26^a	1·23^a	1·02^b	1·07^b	0·93^b	0·05
	6	1·20	1·27	1·21	1·22	1·16	1·23	1·14	0·02
Total lipid (mg g^{-1})	4	271·7^a	268·3^a	240·5^a	242·8^a	98·6^b	89·2^b	91·4^b	30·73
	6	142·1	135·4	132·9	136·8	125·0	121·6	118·8	3·03
Triglyceride (mg g^{-1})	4	195·4^a	195·3^a	161·9^a	159·8^a	30·2^b	26·5^b	28·5^b	28·43
	6	51·6	47·4	44·9	47·3	41·8	40·0	40·5	1·50
Triglyceride (% total lipid)	4	71·9^a	72·8^a	67·3^a	65·8^a	30·6^b	29·7^b	31·2^b	7·34
	6	36·3	35·0	33·8	34·6	33·4	32·9	34·1	0·40

^{a,b} Within a row values followed by different superscript letters are significantly different at $P<0·05$.

TABLE 5
Pyruvate carboxylase activity in livers of broiler chicks

Supplemental biotin ($mg\ kg^{-1}$)	*Enzyme activity* (*units per g liver*)*	
	4 weeks	*6 weeks*
0	$1·4^{c}$	$1·5^{c}$
0·04	$2·5^{c}$	$2·7^{c}$
0·08	$6·3^{bc}$	$6·7^{bc}$
0·12	$10·8^{ab}$	$11·1^{ab}$
0·16	$15·6^{a}$	$14·3^{a}$
0·20	$16·2^{a}$	$14·8^{a}$
0·24	$15·8^{a}$	$14·5^{a}$
±SEM	2·25	2·00

$^{a-c}$ Within a column values followed by different superscript letters are significantly different at $P<0·05$.
* Units expressed as micromoles of oxaloacetate formed per minute at room temperature.

Significant differences in liver and kidney weights (% live weight), lipid fractions in blood and in the two organs, and blood glucose values were observed at the fourth week of age. This therefore confirms the report of Bannister (1976) that FLKS is a metabolic disorder of young chicks. However, pyruvate carboxylase activity in liver was significantly affected by the dietary treatments at the fourth and sixth weeks of study. The significant effect of dietary biotin on pyruvate carboxylase activity at the sixth week of the study was in agreement with the observation of Ogunmodede (1978) thus indicating that birds given 0–0·12 mg biotin per kg feed suffered vitamin deficiency at this age.

Involvement of biotin in abnormal development of leg bone in the chicken has been confirmed by Cook *et al* (1984a,b). Cravens *et al* (1944) observed chondrodystrophy, crooked tibia and shortened or twisted tarsometatarsus in dead embryos from biotin-deficient hens. Couch *et al* (1948) reported that perosis and skeletal deformities developed in embryos and newly hatched chicks when the breeding flock was fed a low-biotin diet. The deformities reported included shortening of the tibiotarsus, which was bent posteriorly, and a much shortened tarsometatarsus. In this trial, leg bone abnormalities developed in experimental broilers within three weeks of the commencement of the study in birds given 0–0·08 mg biotin per kg feed. Crooked, bowed or twisted toes were also found and the majority of affected birds had difficulty in standing or walking. This trial showed that a supplemental biotin level of 0·20 mg kg^{-1} feed was needed to prevent the occurrence of leg deformities in broiler chicks (Table 2).

Information is lacking on the biotin requirement for broiler carcass characteristics such as carcass weight, total bone weight and meat to bone ratio. Results obtained in this study showed that good carcass characteristics required

TABLE 6

Blood glucose, total lipid and free fatty acid concentrations as affected by dietary biotin level

Parameter	*Age (weeks)*	*Supplemental biotin (mg kg^{-1})*							
		0	*0·04*	*0·08*	*0·12*	*0·16*	*0·20*	*0·24*	*±SEM*
Glucose (mg g^{-1})	4	0·58^{b}	0·57^{b}	0·62^{b}	0·61^{b}	0·98^{a}	0·96^{a}	0·97^{a}	0·070
	6	0·79	0·82	0·96	1·00	1·03	1·02	1·02	0·035
Total lipid (mg ml^{-1})	4	21·20^{a}	18·90^{a}	19·10^{a}	16·72^{b}	15·86^{b}	16·02^{b}	15·93^{b}	0·728
	6	23·62	23·12	25·15	24·03	23·71	22·36	23·00	0·310
Free fatty acid (mg ml^{-1})	4	1·02^{a}	0·96^{a}	1·00^{a}	0·99^{a}	0·52^{b}	0·48^{b}	0·46^{b}	0·095
	6	1·26	1·28	1·19	1·07	0·98	1·03	0·96	0·046

^{a,b} Within a row values denoted by different superscript letters are significantly different at $P<0·05$.

biotin as evident from the significant dietary treatment effect on these parameters. It appeared that while 0·16 mg biotin was adequate for good carcass weight, dressing percentage, total edible meat weight and total bone weight, 0·20 mg biotin per kg feed was required for meat and bone weights expressed as a percentage of carcass weight and meat to bone ratio (Table 2).

It may therefore be concluded that broilers given guineacorn/palm kernel meal based diet without biotin supplementation suffered vitamin deficiency, and that a dietary supplemental biotin level of 0·20 mg kg^{-1} feed in such a ration was adequate for the promotion of efficient feed utilisation, good carcass characteristics and prevention of dermal lesions, FLKS mortality and leg deformity.

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