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STUDIES ON THE BIOTIN REQUIREMENT OF BROILERS FED SUNFLOWER SEED MEAL BASED DIETS

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A total of 560 day-old commercial broiler chicks were used in two experiments conducted to investigate the biotin requirement of broilers fed sunflower seed meal (SSM) based diets. Two basal biotin-deficient diets based on dehulled (Experiment 1) and non-dehulled (Experiment 2) SSM were each supplemented with graded levels of biotin so that the rations had 40, 80, 120, 160, 200, 240 and 280 mcg/kg feed. Therefore a total of 14 treatments were tested. Each treatment was given to duplicate floor pens with 20 chicks each for a period of 28 days.

Estimation of the live weights, live weight gain, feed intake, blood glucose and free fatty acid concentrations, lipid contents and weights of liver and kidney, and liver pyruvate carboxylase activity and the records of incidence of dermal lesions, fatty liver and kidney syndrome (FLKS) mortality and leg deformities indicated that in the case of dehulled SSM, dietary biotin of of 160 mcg/kg feed was marginal while at least 200 mcg/kg appeared to be needed for optimum performance of the birds. When non-dehulled SSM was incorporated in the diet, 200 mcg biotin/kg feed was found to be the lowest dietary level needed. However, as it seems that better results could be obtained with higher levels, 240 mcg biotin/kg with non-dehulled SSM is being recommended.

KEY WORDS: Biotin; Broiler chicken; Sunflower seed meal

1. INTRODUCTION

The expansion of Nigerian poultry feed industry is seriously threatened by the high cost and inadequate supply of groundnut cake, a major vegetable protein source in poultry rations. The search for cheap unconventional feed ingredients capable of replacing groundnut cake in poultry diets is therefore inevitable. However, complete replacement of groundnut cake with a lesser — known feed ingredient altered the biotin requirement of broiler chicks (Ogunmodede, 1978; Oloyo, 1991; Oloyo and Ogunmodede, 1991). The difference in the established requirement values was attributed to the high fibre content of the test ingredient.

In recent times, dehulled and non-dehulled sunflower seed meals (SSM) are being used as substitutes for groundnut cake in broiler rations in Nigeria without information on the biotin requirement of broiler chicks fed such rations. Since SSM, especially the non-dehulled form is highly fibrous, it is necessary to

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determine the biotin requirement of broiler chicks fed diets containing such feed ingredient.

Biotin, a micronutrient required for promotion of growth and efficient metabolism of lipids, carbohydrates and protein, and prevention of dermal lesion, fatty liver and kidney syndrome (FLKS) and leg weakness in young chicken (Mistry and Dakshinamurti, 1964; Whitehead and Bannister, 1978; Oloyo and Ogunmodede, 1989), occurs naturally in most feed ingredients but in varying degree of bioavailability to the birds (Wagstaff et al., 1961; Whitehead, 1983; Buenrostro and Kratzer, 1984; Frigg, 1984). Consequently, values reported in the literature as to the biotin requirement of broilers very widely with the different feed ingredients used in the test diets (Wagstaff et al., 1961; Ogunmodede, 1978; Whitehead and Bannister, 1978; Oloyo, 1991; Oloyo and Ogunmodede, 1991).

The objective of the study reported herein therefore was to establish the biotin requirement of commercial broiler chicks fed practical rations containing dehulled or non-dehulled SSM as vegetable protein sources.

2. MATERIAL AND METHODS

2.1. Experiment 1

A total of 280 day-old commercial broiler chicks were randomly assigned to seven treatment groups, the dietary treatments being 40, 80, 120, 160, 200, 240 and 280 mcg biotin/kg feed. Each treatment group had two replicates consisting of 20 birds each. The basal biotin-deficient diet (Table 1) was prepared by formulating a practical diet containing dehulled SSM, and 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 (Wright and Skeggs, 1944) prior to vitamin supplementation. Chicks were raised in 14 floor pens, each of 4.2 m^2 floor area, two 4-litre plastic drinkers, a trough feeder and a 100-W tungsten filament lamp that had guard support around it. Birds were maintained on the respective treatments for a period of 28 days 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 Water Resources for post mortem examination.

Liveweights were measured at 1 and 28 days of age. Feed intake was recorded on a weekly basis. Weight gain and feed conversion (feed intake/gain) were calculated. Weekly record of incidence of dermatitis (percentage of birds within a treatment group showing mild to very severse signs), mortality due to 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 treatment group showing either (i) legs with crooked toes, or (ii) leg with bowed or twisted toes, or (iii) difficulty in standing or walking were kept.

Table 1 Composition of basal biotin deficient diets

Constituent	*Inclusion level (g kg^{-1})*	
	Experiment 1	*Experiment 2*
Yellow maize	630	650
Sunflower seed meal (A)*	200	—
Sunflower seed meal (B)*	—	200
Blood meal	32	46
Fish meal	35	31
Brewer's grain	50	40
Oyster shell	20	10
Bone meal	30	20
Vitamin/mineral premix*	1	1
Salt (NaCl)	2	2
Analysis		
Biotin (mcg kg^{-1})	36.1	36.8
Calculated		
Crude protein (%)	21.2	21.0
Metabolizable energy, ME (MJ/kg)	12.13	11.99
Crude fibre (%)	3.1	5.2

*Vitamin/mineral premix supplied the following vitamins and mineral elements per kg of feed: Vitamin A, 1200 i.u; Vitamin D, 2500 i.u; Vitamin E, 10 i.u; Menadione sodium bisulphite, 1.5 mg; Vitamin B_1, 2.5 mg; Vitamin B_2, 5mg; Cholin chloride, 500 mg; Calcium d-panthothenate, 10mg; Nicotinic acid, 35mg; Vitamin B_6, 4mg; Vitamin B_{12}, 0.02mg; Iron, 50mg; Manganese, 150mg; Copper, 2.5mg; Zinc, 45mg; Cobalt, 0.2mg; Selenium, 0.08mg; Iodine, 1.4mg. +A, Dehulled; B, Non-dehulled

At the end of the feeding trial, four replicate samples (birds) were randomly selected from the respective treatment groups for blood collection and subsequent slaughtering. 2 ml of blood sample was taken from the wing veins of each bird into heparinized containers and kept for the estimation of glucose and free fatty acid concentrations by the methods of Dubois et al. (1956) and Pearson (1976) respectively. Selected bird samples were slaughtered, the liver and kidneys were excised, drained of fluid with blotting paper and weighed. These organs were freeze — dried and kept for estimation of total lipid by the method of Folch et al. (1957) and triglyceride as described by Fletcher (1968). Pyruvate carboxylase activity in livers were also measured in accordance with the procedure of Utter and Keech (1963).

2.2 Experiment 2

In this study, non-dehulled SSM completely replaced dehulled SSM in the biotin-deficient basal diet of experiment 1 (Table 1). Consequently, biotin-deficient diet containing non-dehulled SSM constituted the basal diet for the second experiment, and thus supplemented with graded levels of biotin such that experimental diets had 40, 80, 120, 160, 200, 240 and 280 mcg of the vitamin per kilogramme of feed. Experimental diets were fed to duplicate groups of day-old broiler chicks with 20 birds per group (40 birds per treatment) for a period of 28 days. Experimental birds were reared in 14 floor pens and the management practise adopted was as described in the previous experiment. Record of live weight,

weight gain, feed intake, feed conversion, and incidence of dermatitis, FLKS mortalilty and leg deformities were kept as indicated in the first study. As the 28th day, four birds were randomly removed from each of the treatment groups for blood collection and subsequent slaughtering. Blood, liver and kidney were analysed for biochemical parameters as specified in experiment 1.

2.3 Statistical Analysis

Results obtained in the two studies were subjected to statistical analysis in accordance with the procedures of Steel and Torrie (1960). Treatment means that differed significantly were separated by the multiple range test of Duncan (1955).

3. RESULTS AND DISCUSSION

In an attempt to estimate the biotin requirement of broiler chicks fed either dehulled or non-dehulled SSM based diets, birds were given basal diets (Table 1) supplemented with graded levels of the vitamin so that a wide range of biotin level was spanned. The lowest dietary biotin level (40 mcg/kg feed) was the vitamin level used to reproduce FLKS, a deficiency symptom of biotin in chicken (Oloyo and Ogunmodede, 1989). The highest dietary biotin level (280 mcg/kg feed) was seven times the lowest, and was more than estimated requirement values of 120 mcg/kg (Ogunmodede, 1978). 160 mcg/kg (Whitehead and Bannister, 1980; Oloyo and Ogunmodede, 1989) and 200 mcg/kg (Oloyo, 1991) reported in the literature.

Growth and feed conversion of broiler chicks are given in Table 2. While live weight at 28 days of age, live weight gain and feed intake were significantly affected by the dietary treatments, feed conversion was not. Within the groups of birds that received diets containing dehulled SSM, those on up to 120 mcg/kg consumed significantly less feed, had poorer liveweight gain and weighed less at 28 days than those given 160–180 mcg/kg. On the other hand, birds on up to 160 mcg/kg of non-dehulled SSM based diets had poorer growth performance than those on 200–280 mcg/kg. Poor feed consumption observed in birds given 40–120 mcg biotin/kg dehulled SSM and 40–160 mcg biotin/kg non-dehulled SSM might be due to reduction in free movement of the birds as a result of abnormal development of leg bones as well as the dermal lesions developed in the feet.

Results from this study tends to show that whereas 160 mcg biotin/kg was the lowest dietary level required for optimum feed intake and growth rate when dehulled SSM was used in the diet, 200 mcg/kg was the minimum biotin requirement when non-dehulled SSM based diet was fed.

Incidence of biotin deficiency signs in experimental broiler chicks are given in table 3. Foot dermatitis, a biotin deficiency symptom described by Frigg et al (1973), Ogunmodede (1978) and Oloyo (1991) developed in birds that received dietary biotin levels of 40 to 160 mcg/kg and 40 to 200 mcg/kg in diets containing dehulled SSM and non-dehulled SSM respectively.

Table 2 Growth and feed conversion of broiler chickens which received either dehulled (A) or non-dehulled (B) sunflower seed meal (SSM) and graded levels of biotin from 1 to 28 days of age

SSM	Biotin mcg kg^{-1}	Live weight at 28 days (g)	Live weight gain (g/bird·day)	Feed intake (g/bird·day)	Feed conversion (g feed/g gain)
A	40	436.9d*	14.1c	40.2d	2.85
	80	455.8bc	14.8bc	39.9d	2.70
	120	475.3bc	15.5bc	44.1cd	2.85
	160	581.3a	19.3a	55.5a	2.88
	200	569.4a	18.8a	52.9ab	2.81
	240	576.6a	19.1a	54.2ab	2.84
	280	578.2a	19.2a	54.6a	2.84
B	40	372.4e	11.8d	30.7e	2.60
	80	380.2e	12.1d	33.4e	2.76
	120	423.6de	13.6cd	38.8d	2.85
	160	445.5cd	14.4c	40.3d	2.80
	200	507.4b	16.6b	48.1bc	2.90
	240	512.8b	16.8b	47.7bc	2.84
	280	510.0b	16.7b	47.9bc	2.87
	±SEM**	18.56	8.63	2.05	0.021

* Mean values in a column followed by different subscripts are significantly different at $P<0.05$
**SEM, standard error of the mean

Incidence of dermatitis was higher (13.9%) in birds given non-dehulled SSM than those fed dehulled SSM based diets (7.9%). Affected birds showed varying degree of swollen foot pad with haemorrhagic fissures. Cracked and swollen areas occurred on pads on the bottom of the feet. Haemorrhagic cracks developed around the metatarsal area of the foot pads. Previous studies have shown that while 120 mcg/kg (Ogunmodede, 1978) and 200 mcg/kg (Oloyo, 1991) prevented dermal lesions in broiler chicks, Whitehead and Bannister (1978) noted that the severity of foot lesions was only reduced by increasing the supplemental biotion level but the lesions were not eliminated by 500 mcg/kg, the highest level tested. The result obtained in this trial showed that 200 mcg/kg was required to prevent desmal lesions in broiler chicks when fed dehulled SSM based diet, whereas 240 mcg/kg was required in case of non-dehulled SSM based diet.

FLKS, a biotin deficiency symptom in young chicken usually in the age range of 3 to 5 weeks is characterised by morbidity followed by death within a few hours. The mortality can occur as early as 10 days and as late at 56 days of age and its incidence can be eliminated by dietary biotin supplementation (Payne et al. 1974; Bannister, 1976; Whitehead et al., 1976).

Record of mortality due to FLKS is given in Table 3. Diets based on non-dehulled SSM caused 7.1% mortality, but dehulling of the SSM resulted in a marked decrease (3.9%). Supplementation of diets based on either dehulled or non-dehulled SSM with biotin significantly affected the incidence of FLKS mortality among the experimental birds, and that the lowest level that prevented

Table 3 Incidence of dermatitis, FLKS mortality and leg deformities in broiler chickens fed diets containing either dehulled (A) or non-dehulled (B) sunflower seed meal (SSM) and graded levels of biotin from 1 to 28 days of age

SSM	*Biotin (mcg kg^{-1})*	*Dermatitis (%)*	*FLKS mortality (%)*	*Leg deformities (%)*
A	40	30.0b*	12.5ab	10.0b
	80	15.0c	10.0b	7.5c
	120	7.5d	5.0c	7.5c
	160	2.5e	0.0d	2.5d
	200	0.0e	0.0d	0.0d
	240	0.0e	0.0d	0.0d
	280	0.0e	0.0d	0.0d
B	40	45.0a	15.0a	15.0a
	80	32.5b	15.0a	12.5ab
	120	15.0c	10.0b	7.5c
	160	2.5e	10.0b	7.5c
	200	2.5e	0.0d	2.5d
	240	0.0e	0.0d	0.0d
	280	0.0e	0.0d	0.0d
	±SEM**	3.81	1.60	1.32

* Mean values in a column followed by different subscripts were significantly different at $P<0.05$
** SEM, Standard error of the mean

FLKS mortality were 160 mcg/kg and 200 mcg/kg for diets based on dehulled and non-dehulled SSM respectively.

Characteristically, FLKS — affected chicken show elevated blood total lipid and fatty acid concentrations, and exhibited markedly reduced glucose level (Bannister et al., 1975; Balnave et al., 1977; Oloyo and Ogunmodede, 1991). Whitehead et al. (1976) and Oloyo (1991) also noted that the primary abnormality in the FLKS condition is the failure of hepatic gluconeogenesis due to reduced pyruvate carboxylase activity, a biotin — dependent enzyme. Consequently, severe hypoglycaemia which is believed to be the cause of death results. Results presented in Table 4 indicated that experimental chicks given 40–80 mcg biotin/kg dehulled SSM and those given 40–120 mcg/biotin/kg non-dehulled SSM had significantly higher blood free fatty acid concentration but had lower liver pyruvate carboxylase activity and lower blood glucose concentrations.

This result indicated that the affected birds suffered hypoglycaemia, hence the higher FLKS mortality recorded among these groups (Table 3).

Chemical analysis of the liver and kidney of FLKS — affected chicken revealed that these organs were enlarged and markedly involved in fatty infiltration (Whitehead et al., 1975). Johnson et al. (1972), Whitehead (1975) and Oloyo (1991) noted that the extra lipid deposited in the organs was mainly triglyceride. Results obtained in this study is in agreement with the report of earlier workers. Significantly higher liver and kidney weights as well as higher lipid contents in birds fed 40–120 mcg biotin/kg dehulled SSM based diets and those given

Table 4 Blood glucose and free fatty acid concentrations, and liver pyruvate carboxylase activity in experimental broiler chickens at 28 days of age

SSM	*Biotin (mcg kg^{-1})*	*Glucose (mmole l^{-1})*	*Free fatty acid (mmole l^{-1})*	*Liver pyruvate carboxylase activity (units/g liver)*+
A	40	3.81b*	3.21a	2.7c
	80	3.86b	3.16a	6.3b
	120	5.28a	1.70b	12.8a
	160	5.36a	1.81b	13.6a
	200	5.58a	1.88b	14.1a
	240	5.50a	1.81b	14.4a
	280	5.53a	1.74b	14.2a
B	40	3.53b	3.48a	2.5c
	80	3.83b	3.26a	5.5b
	120	4.19b	3.09a	6.4b
	160	5.36a	1.74b	14.2a
	200	5.22a	1.91b	14.1a
	240	5.42a	1.77b	14.3a
	280	5.40a	1.70b	13.9a
	±SEM**	0.204	0.188	1.22

* Mean values in a column followed by different subscripts differ significantly at $P<0.05$
** SEM, Standard error of the mean
+ Units expressed as micromoles of oxaloacetate formed per mintue at room temperature

40–120 mcg/kg non-dehulled SSM based diets (Table 5) reflected fatty infiltration of the two organs due to biotin deficiency, hence the higher incidence of mortality due to FLKS.

Frigg et al. (1973) and Cook et al. (1984 a, b) confirmed the involvement of biotin in abnormal development of leg bone in young chicken and turkeys. Earlier reports by Cravens et al. (1944) and Couch et al. (1948) indicated that skeletal deformities, perosis, chondrodystrophy, crooked tibia and shortened or twisted tarsometatarsus were observed in dead embryos and newly hatched chicks when the breeding flock was fed a biotin — defiicient diet. In this study, leg bone abnormalities developed in experimental chicks fed 40–160 mcg biotin/kg dehulled SSM based diets, and those fed 40–200 mcg biotin/kg non-dehulled SSM based diet (Table 3). Throughout the period of study, incidence of leg deformities recorded in groups of birds fed dehulled SSM and non-dehulled SSM based diets were 3.9% and 6.4% respectively. Crooked, bowed or twisted toes were pronounced in affected birds with majority of them having difficulty in standing or walking.

From the foregoing therefore, it may be concluded that to keep at minimum level the incidence of dermal lesion, FLKS mortality and leg bone deformities and promote good feed utilisation, 160 mcg biotin/kg feed was required when dehulled SSM was fed to broilers, however 200 mcg biotin/kg feed was needed when non-dehulled SSM constituted the vegetable protein source in diets of broilers. Moreover higher levels of the vitamins (i.e. 200 mcg/kg with dehulled SSM and 240 mcg/kg with non-dehulled SSM) were required to prevent the

Table 5 Weight and lipid content of liver and kidney of broiler chicken fed diets containing dehulled (A) and non-dehulled (B) sunflower seed meal (SSM) and graded levels of biotin at 28 days of age

SSM	Biotin (mcg kg^{-1})	Liver Weight (g)	Liver Total lipid (mg g^{-1})	Liver Triglyceride (mg g^{-1})	Kidney Weight (g)	Kidney Total lipid (mg g^{-1})	Kidney Triglyceride (mg g^{-1})
A	40	19.59a*	286.4ab	206.2a	8.29a	214.8ab	154.7a
	80	19.09a	262.7abc	188.6abc	8.46a	204.9ab	145.9a
	120	18.83a	238.0c	150.2c	7.81a	168.9b	117.6a
	160	16.40b	164.2d	73.9d	5.87b	90.3c	27.1b
	200	15.94b	158.1d	72.6d	5.60b	83.8c	24.2b
	240	16.30b	162.2d	76.7d	5.56b	87.6c	27.2b
	280	16.20b	162.3d	74.5d	5.74b	82.8c	26.5b
B	40	20.10a	294.4a	216.7a	8.82a	228.7a	157.1a
	80	19.82a	287.6ab	199.6ab	8.24a	213.4ab	138.5a
	120	20.12a	275.8abc	178.7abc	8.05a	190.9ab	133.6a
	160	18.75a	242.1bc	152.0bc	7.75a	178.2ab	121.2a
	200	16.40b	159.9d	73.9d	4.82b	83.2c	25.5b
	240	16.23b	161.2d	75.4d	5.01b	87.1c	26.1b
	280	16.30b	160.3d	73.3d	4.89b	85.6c	24.9b
	±SEM**	0.443	15.02	15.42	0.393	15.73	15.27

* Means in a column followed by different subscripts differ significantly at $P<0.05$
** SEM, Standard error of the mean

incidence of biotin deficiency symptoms in experimental broilers.

Experimenting with broiler chicks, Misir and Blair (1984) noted that inclusion of fibre in the diet reduced the bioavailability of biotin from cereal grains such as corn, sorghum, wheat and triticale. It was also suggested that dietary fibre might be interfering with the absorption of the free biotin from the bird's gut. The result obtained in this study tended to suggest that a difference of 2.1% crude fibre content between the diets based on dehulled and non-dehulled SSM (Table 1) was sufficient to produce a marked difference in the biotin requirement of the two groups of experimental chicks.

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Dehulling of SSM before inclusion in the diets of broilers is therefore beneficial because it reduces the amount of vitamin required as well as minimize losses that otherwise will result from high incidence of dermal lesions, leg deformities and FLKS mortality.

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