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The biotin requirement of broilers feed maize-palm kernel meal based ration

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6 duplicate floor pens with 20-day-old commercial broiler chicks each, were fed practical-type broiler diet with maize-palm kernel meal, supplemented with graded levels of biotin so that the rations had 40, 80, 120, 160, 200, and 240 µg of the vitamin per kg of feed. This treatment was maintained for 6 weeks. Estimation of the body weight, feed intake, blood glucose concentration, and lipid contents of liver, kidney and blood showed that dietary biotin of 120 µg/kg feed is a minimum requirement. 160 µg/kg feed was required for the prevention of dermatitis, mortality due to fatty liver and kidney syndrome (FLKS) and leg deformities.

1. Introduction

The ever-increasing cost of feed poses a maior threat to the development of poultry industry in Nigeria. This problen has led to a search for alternative feed ingredient sources for poultry diets. However, substitution of unconventional ingredients for conventional ones in diets of birds may alter the micronutrient requirement of the bird.

While the use of palm kernel meal in poultry rations as a substitute for more costly and expensive conventional feedstuffs is becoming increasingly popular in Nigeria, there are still some unanswered questions as to the biotin requirement of broiler chicks fed rations based on maize-palm kernel meals. It has been reported that the inclusion of fibre in the diet of broiler chicks 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 free biotin from the gut (MISIR and BLAIR 1984). Availability of phosphorus, magnesium, iron, zinc, and copper was depressed by crude fibre content of the chick diet (NWOKOLO and BRAGG 1977). Since palm kernel meal is noted for its high fibre content, it is therefore necessary to determine the biotin requirement of broiler chicks fed palm-kernel meal based rations.

Another factor that must be taken into consideration when changing or substituting, ingredients in rations for poultry is the bioavailability of micronutrients present in such ingredients, especially since available biotin can be much less than the total analysed amounts in various

feedstuffs (WAGSTAFF et al. 1961, ANDERSON and WARNICK 1970, WHITEHAD et al. 1974, FRIGG 1976, 1984). For broilers it was observed that while 120 µg/kg feed was the minimum needed in maize-groundnut cake, a level of 150 µg/kg feed was adequate for guineacorn-groundnut cake ration (OGUNMODEDE 1978). Lower available biotin contents than the total analysed amounts in broiler diets based on combinations of some commonly available ingredients such as maize, sorghum, wheat, soybean meal, fish, and meat meal were observed (WHITEHEAD 1983). The total available biotin was far below the level recommended by the Agricultural Research Council (1975), so that there was a need for supplementation. This seems to suggest that the biotin requirement of broilers varies with different diets as some of the ingredients change in proportion or are completely substituted.

In this study, palm-kernel meal was used as a plant protein source in the formulation of practical rations for commercial broilers with a view to establishing the biotin requirement of broilers fed such rations.

2. Experimental

2.1. Birds, diets, and husbandry

Duplicate groups of day-old commercial broiler chicks with 20 chicks per group (40 chicks per treatment) were given basal biotin-deficient diet (table 1) supplemented with graded levels of biotin, so that the experirnental rations had 40, 80, 120, 160, 200, and 240 µg biotin per kg of feed. The broiler chicks were housed in 15 deep-litter pens (4.1 m^2), 20 birds per pen, and they had free access to feed and water at all times. Each pen was heated with a 100 watt tungsten lamp that had guard support arround it. The experimental birds were kept on these treatments for 6 weeks. All birds that died were sent to the Faculty of Veterinary Medicine, University of Ibadan, for *post mortem* examination.

Weekly records were made of the feed intake, body weight, 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 treatment group showing legs with crooked toes, or legs with bowed or twisted toes, or difficulty in standing or walking.

At the fourth and sixth weeks, 2 ml of blood were collected in heparinized containers from wing veins of each of 4 replicate samples of experimental chicks randomly selected from each treatment group for the determination of glucose, total lipid, and free fatty acid contents. Selected bird samples were then slaughtered, the livers and kidneys were excised, drained of fluid with blotting paper, and weighed. These organs were freeze-dried, and kept for subsequent estimation of total lipid and triglyceride contents.

2.2. Analytical procedure

- Blood samples were deproteinized by the addition of barium hydroxide and zinc sulphate solutions prior to the estimation of glucose (DUBOIS et al. 1956).
- Total lipid contents were determined by the method of FOLCH et al. (1957).

- Triglyceride content was estimated as described by FLETCHER (1968).
- Free fatty acid content was determined by the method of PEARSON (1976.)

Table 1: Composition of basal biotin-deficient ration (%)

Yellow maize	54.0
Palm-kernel meal	18.0
Blood meal	10.0
Fish meal	2.5
Brewer's grain	12.2
Oyster shell	1.0
Bone meal	2.0
Vitamin/mineral premix (UNI-VIT 15)*	0.1
Salt (NaCl)	0.2
Calculated analysis	
Crude protein (%)	21.26
Metabolizable energy, ME Kcal·kg^{-1}	2741.92
ME/CP	128.73
Fat (%)	3.50
Linoleic acid (%)	1.40
Biotin (μ/kg)	38.90

*UNIT-VIT 15 supplied the following vitamins and mineral elements per kilogram of feed: Vitamin A, 8000 i.u.; Vitamin D_3, 1500 i.u.; Vitamin E, 3 i.u., Menadione sodium bisulphite (vitamin K), 1.5 mg; Vitamin B_2, 2.5 mg; Calcium d-pantothenate, 3 mg; Nicotinic acid, 8 mg; Vitamin B_6, 0.3 mg; Vitamin B_{12}, 0.008 mg; Iron. 15 mg; Manganese, 25 mg; Copper, 2.5 mg; Zinc, 10 mg; Iodine, 0.3 mg

2.3. Statistical analysis

The results obtained were subjected to an analysis of variance in accordance with procedures of STEEL and TORRIE (1960). Significantly different treatment means were separated by the multiple range test of DUNCAN (1955).

3. Results and discussion

In order to establish the biotin requirement of broiler chicks fed maize- and palm-kernel meal based ration, birds were given a basal biotin-deficient diet (table 1) supplemented with graded levels of the vitamin. The lowest dietary level (40 μg/kg) 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 (1978a), respectively. The highest dietary biotin level (240 μg/kg feed) was 6 times the lowest.

Feed utilization and biotin-related features in the experimental chicks are shown in table 2. While feed intake and body weight of broiler chicks were significantly influenced by dietary biotin treatments, feed efficiency was not. Birds given 40 and 80 μg/kg feed consumed significantly less feed and had a smaller body weight than those given 120-240 μg/kg feed. The reduced feed consumption observed in birds given 40 and 80 μg/kg 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 seemed to suggest that up to 80 μg/kg feed was not adequate for efficient feed utilization.

Foot dermatitis characteristic of biotin deficiency (PATRICK et al. 1942, FRIGG et al. 1973) developed distinctly as early as the first week of feeding in birds given 80 μg/kg feed and as late as the fourth week in those given 200 μg/kg. No incidence was observed in those given 240 μg/kg throughout the period of study. The food pads were swollen and contained haemorrhagic fissures. Cracked and swollen areas occurred on pads on the bottom of the feet. These lesions varied in severity as shown by the varying degrees of haemorrhagic cracks and sizes of callouses developed on the foot pads especially around the metatarsal area. While it was reported that 120 μg/kg feed prevented dermal lesions in broiler chicks (OGUNMODEDE 1978), some other workers (WHITEHEAD and BANNISTER 1978b) reported that the severity of foot lesions was only reduced by increasing the level of biotin supplementation but were not eliminated at the highest level studied (500 μg/kg feed). In this study, the percentage incidence of dermatitis showed that up to 120 μg/kg feed did not significantly reduce the incidence of the lesion in the affected birds (table 2).

Mortality due to fatty liver and kidney syndrome (FLKS), a biotin deficiency symptom in chicken (HEMSLEY 1965, RIDDELL et al. 1971, PAYNE et al. 1974, WHITEHEAD and BLAIR 1974) was recorded in this study among chicks given 40-120 μg/kg feed (table 2). FLKS, a metabolic disorder, is characterized by morbidity followed by death in young chicks, usually in the age range of 3 to 5 weeks, but it can occur as early as 10 days and as late as 56 days of age (BANNISTER 1976). Its incidence can be eliminated by biotin supplementation in the diet (PAYNE et al. 1974, WHITEHEAD et al. 1976). In this study FLKS mortality was recorded as early as the first week of feeding in birds given 80 and 120 μg/kg feed, and as late as the fifth week in those given 80 μg/kg feed. No death due to FLKS was recorded in the sixth week of study. The percentage FLKS mortality showed that up to 120 μg/kg feed did not prevent the incidence of the syndrome.

Histological examination (WHITEHEAD et al. 1973, WIGHT and SILLER 1975) as well as chemical analysis (WHITEHEAD 1975) of the liver and kidney of FLKS-affected chicken revealed that these organs were enlarged and markedly involved in fatty infiltration. Significantly higher liver and kidney weight as proportion of the live weight as well as significantly higher lipid contents of the two organs in broiler chicks fed 40 and 80 μg/kg feed (tables 3 and 4) reflected the deposition of fat in these organs as a result of biotin deficiency; hence a higher incidence of FLKS mortality was recorded. The triglyceride content and triglyceride of the total lipid in the two organs were significantly higher in broiler chicks given 40 and 80 μg/kg feed than those given higher dietary biotin levels (120-240 μg/kg feed) (tables 3 and 4). This is in agreement with the findings of JOHNSON et al. (1972) and WHITEHEAD (1975), who noted that the extra lipid in liver and kidneys of FLKS chicken was mainly triglyceride.

Elevated level of plasma-free fatty acid and markedly reduced glucose level were observed in FLKS-affected chicken (WHITEHEAD et al. 1973, BANNISTER et al. 1975, BALNAVE et al. 1977). It was also indicated that the primary abnormality in the FLKS condition is a failure of hepatic gluconeogenesis via pyruvate carboxylase, a biotin-dependent enzyme, and this results in severe hypoglycaemia, which was believed to be the cause of death (WHITEHEAD et al.1976). In this study, birds given 40 and 80 μg/kg feed had significantly higher blood-free fatty acid and blood-free fatty acid as percengage of total lipid, but had a significantly lower blood glucose concentration (table 5). This observation indicated a hypoglycaemia condition; hence the higher incidence of FLKS mortality recorded in these groups of birds. In effect, the liver, kidney, and blood lipid as well as blood glucose values suggested that the 120 μg/kg feed was marginal.

Table 2: Feed utilization and biotin-related features in broilers fed graded levels of the vitamin*

Parameter	Age (weeks)	Dietary biotin levels (μ/kg feed)					
		40	80	120	160	200	240
		Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd
Feed intake (g)	4	279.5b±55.9	284.0b±33.2	322.0a±67.9	318.0a± 9.9	365.0a±28.3	334.5a± 7.8
	6	332.0b±58.0	439.5b±97.3	532.0a±61.2	468.0a±62.3	524.5a±65.8	522.5a± 7.8
Body weight (g)	4	252.3b± 9.2	266.5b±12.0	310.0a±14.1	297.5a±10.6	297.5a±10.6	300.0a±10.0
	6	415.4b± 6.4	435.7b± 9.9	550.0a± 2.8	535.0a± 7.1	534.5a±81.3	530.0a± 2.1
Feed efficiency (Wt gained/feed intake)	4	0.20± 0.01	0.25± 0.01	0.25± 0.07	0.22± 0.03	0.24± 0.07	0.24± 0.01
	6	0.19± 0.01	0.21± 0.01	0.15± 0.01	0.17± 0.01	0.20± 0.11	0.17± 0.15
Incidence of dermatitis (%)	2	20.0a	17.5b	0.0c	0.0c	0.0c	0.0c
	4	60.0a	45.0b	10.0c	2.5d	2.5d	0.0d
	6	70.0a	45.0b	10.0c	2.5d	2.5d	0.0d
FLKS mortality (%)	2	5.0a	5.0a	5.0a	0.0b	0.0b	0.0b
	4	10.0a	7.5b	5.0c	0.0d	0.0d	0.0d
	6	10.0a	10.0a	5.0b	0.0c	0.0c	0.0c
Incidence of leg deformities (%)	2	0.0	0.0	0.0	0.0	0.0	0.0
	4	7.5a	5.0b	2.5c	0.0d	0.0d	0.0d
	6	12.5a	7.5b	5.0b	0.0c	0.0c	0.0c

*Values denoted by different subscripts in a row were significantly different at P (0.05).

Table 3: Weights and lipid contents of liver from broilers fed graded levels of biotin*

Parameter	Age (weeks)	Dietary biotin levels (µg/kg feed)					
		40	80	120	160	200	240
		Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd
Weight (g)	4	9.56 ± 0.67	9.11 ± 0.93	8.29 ± 0.19	7.90 ± 0.96	8.00 ± 1.41	7.65 ± 0.89
	6	16.06 ± 4.25	13.97 ± 1.80	15.54 ± 3.96	15.71 ± 2.43	15.74 ± 2.76	15.59 ± 3.08
Weight (% of live wt)	4	3.31a± 0.14	3.33a± 0.41	2.70b± 0.06	2.59b± 0.24	2.78b± 0.41	2.73b± 0.32
	6	3.44 ± 0.92	3.53 ± 0.57	3.15 ± 0.78	3.18 ± 0.43	3.08 ± 0.58	3.46 ± 0.76
Total lipid ($mg \cdot g^{-1}$)	4	299.14a± 8.32	254.14b± 7.71	169.95c±10.04	160.95c± 9.59	163.05c±23.18	159.53c±18.97
	6	179.74 ±33.63	169.09 ±20.31	171.23 ±38.45	169.82 ±15.00	169.28 ±13.77	165.43 ±17.89
Triglyceride ($mg \cdot g^{-1}$)	4	210.33a± 7.08	165.45b± 5.41	79.87c± 3.71	73.96c± 6.04	75.29c±11.72	74.71c± 9.49
	6	86.44 ±11.45	81.06 ±11.03	80.41 ±19.27	78.27 ± 8.16	81.07 ± 9.99	77.75 ±11.61
Triglyceride (% of total lipid)	4	70.28a± 1.67	65.10b± 1.08	47.02c± 0.61	45.91c± 1.51	46.11c± 0.80	46.81 ± 0.89
	6	48.45 ± 3.10	47.90 ± 1.46	46.85 ± 2.36	46.09 ± 2.68	47.78 ± 2.26	46.82 ± 2.23

*Values denoted by different subscripts in a row were significantly different at P (0.05).

Table 4: Weight and lipid contents of kidneys from broilers fed graded levels of biotin*

Parameter	Age (weeks)	Dietary biotin levels (μg/kg feed)					
		40	80	120	160	200	240
		Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd
Weight (g)	4	3.56 ± 0.31	3.51 ± 0.37	3.09 ± 0.09	3.14 ± 0.40	3.13 ± 0.09	2.96 ± 0.73
	6	5.76 ± 0.35	5.63 ± 0.92	5.47 ± 0.89	5.60 ± 1.55	6.02 ± 1.33	5.56 ± 1.85
Weight (% of live weight)	4	1.21a± 0.09	1.29a± 0.17	1.01b± 0.03	1.03b± 0.14	1.09b± 0.08	1.12b ± 0.15
	6	1.28 ± 0.16	1.44 ± 0.39	1.11 ± 0.17	1.14 ± 0.30	1.16 ± 0.15	1.21 ± 0.30
Total lipid ($mg \cdot g^{-1}$)	4	349.46a±37.01	332.55a±29.23	189.73b±13.87	196.28b±26.13	187.39b±11.37	196.01b ±36.22
	6	201.58 ±10.33	203.80 ±28.90	211.31 ±36.05	215.49 ±50.11	198.47 ±48.06	219.77 ±66.72
Triglyceride ($mg \cdot g^{-1}$)	4	247.91a±22.36	225.14b±20.98	60.01c± 5.29	63.09c± 9.37	56.05c± 2.58	60.51c ±11.68
	6	62.72 ± 3.70	57.89 ± 8.61	62.02 ±10.45	61.18 ±17.68	56.84 ±14.82	66.44 ±21.31
Triglyceride (% of total lipid)	4	70.93a± 1.26	67.68b± 0.50	31.62c± 1.38	32.10 ± 0.54	29.92d± 0.36	30.83cd± 0.61
	6	31.11 ± 0.52	28.38 ± 0.24	29.36 ± 0.37	28.11 ± 1.64	28.56 ± 0.78	30.10 ± 0.69

*Values denoted by different subscripts in a row were significantly different at P (0.05).

Table 5: Influence of dietary biotin levels on the blood glucose, total lipid, and free fatty acid contents*

Parameter	Age (weeks)	Dietary biotin levels (µg/kg feed)					
		40	80	120	160	200	240
		Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd
Glucose ($mg \cdot ml^{-1}$)	4	0.61d±0.05	0.63cd±0.08	0.69cd±0.04	0.78bc±0.10	0.89ab±0.08	0.94a±0.12
	6	1.08 ±0.11	1.15 ±0.07	1.20 ±0.13	1.10 ±0.06	1.21 ±0.14	1.20 ±0.17
Total lipid ($mg \cdot ml^{-1}$)	4	19.90 ±2.45	17.07 ±3.35	16.20 ±3.59	15.65 ±1.45	16.10 ±3.96	16.60 ±1.36
	6	30.63 ±4.00	30.38 ±4.41	29.50 ±3.62	29.75 ±1.92	30.00 ±3.59	29.88 ±1.82
Free fatty acid ($mg \cdot ml^{-1}$)	4	0.90a±0.10	0.74ab±0.08	0.53b ±0.24	0.46b ±0.07	0.51b ±0.19	0.46b±0.15
	6	2.01 ±0.62	1.50 ±0.43	1.09 ±1.25	0.92 ±0.63	1.00 ±0.18	1.03 ±0.30

*Values denoted by different subscripts in a row were significantly different at P (0.05).

Biotin, among other vitamins and trace minerals, caused leg bone abnormalities in broilers (COOK et al. 1984a,b). Chondrodystrophy, crooked tibia, and shortened or twisted tarsometatarsus were observed in dead embryos from biotin-deficient hens (CRAVENS et al. (1944). Perosis and characteristic skeletal deformities developed in embryo and newly-hatched chicks when the breeding flock was fed a low-biotin diet (COUCH et al. 1948). The deformities reported included shortening of the tibiotarsus which was bent posteriorly, and a shortened tarsometatarsus. In this study, leg bone abnormalities developed in broilers within 3 weeks of study in birds given 40 to 120 µg/kg feed. Crooked, bowed, or twisted toes caused difficulties in standing or walking for the majority of affected birds.

The results in table 2 show that up to 120 µg/kg feed was not adequate to prevent the incidence of leg deformity in broiler chicks. It may therefore be concluded that a dietary biotin level of 160 µg/kg feed was adequate for the promotion of good feed utilization and the prevention of dermatitis, FLKS mortality, and leg deformity in areas where maize and palm-kernel meal are used as feed ingredients for broiler rations.

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R.A. OLOYO und B.K. OGUNMODEDE: Biotinbedarf von Broilern, die mit Rationen auf Mais-Palmkernmehl-Basis gefüttert wurden

Je 20 handelsübliche Eintagsbroilerküken in 6 doppelten Abteilungen mit Bodenaufstallung erhielten landesübliches Broilerfutter aus Mais-Palmkernmehl, das abgestuft mit Biotin ergänzt wurde, so daß die Rationen 40, 80, 120, 200 und 240 µg dieses Vitamins je kg Futtermittel enthielten. Die Broiler wurden für die Dauer von 6 Wochen der jeweiligen Fütterung unterzogen, wobei die Entwicklung der Körpermasse, der Futteraufnahme, der Blutzuckerkonzentration, des Fettgehaltes von Leber, Niere und des Blutes zeigten, daß ein Biotingehalt von 120 µg/kg Futtermittel dem Minimalbedarf zu entsprechen scheint. 160 µg/kg Futtermittel waren das Gehaltsniveau, das dem Auftreten von Dermatitis, Todesfällen im Zusammenhang mit dem Fettleber- und Fettnierensyndrom (FLKS) sowie Beindeformationen vorbeugte und daher empfohlen wird.

R.A. OLOYO et B.K. OGUNMODEDE: Les besoins en biotine des poulets rôtis nourris à base de farine de noyaux de palme et maïs

20 poussins rôtis usuels d'un jour ont reçu chacun, dans 6 compartiments doubles avec logement au sol, de la nourriture à poulets, telle qu'elle est en usage dans le pays, c'est-à-dire, à base de farine de noyaux de palme et maïs, laquelle a été complétée de façon échelonée avec de la biotine de sorte que les rations contenaient 40, 80, 120, 160, 200 et 240 µg de cette vitamine par kg de la nourriture. Les poulets ont reçu pour la durée de six semaines la nourriture respective, et le développement de la masse du corps, de l'absorption de nourriture, de la concentration de la glycémie, de la teneur en graisse du foie, de reins et du sang montraient qu'une teneur en biotine de 120 µg/kg de nourriture semble correspondre aux besoins minima. 160 µg/kg de nourriture était le niveau de teneur qui prévenait la dermatite, les cas mortels en rapport avec le syndrome du foie gras et des reins gras (FLKS) ainsi que les déformations des jambes et qui est, par conséquent, recommandé.

R.A. OLOYO y B.K. OGUNMODEDE: Necesidad de biotina en polluelos de engorde alimentados con raciones a base de maíz y harina de pepitas de palma

20 polluelos comerciales de 1 día de edad criados en 6 secciones dobles al suelo recibieron la comida usual compuesta de maíz y de harina de pepitas de palma que se completó con biotina de modo que las raciones contenían 40, 80, 120, 160, 200 y 240 µg de esta vitamina. Los polluelos fueron alimentados durante 6 semanas con la correspondiente comida y el desarrollo de la masa corporal, la absorción de la comida, la concentración del azúcar sanguíneo, el contenido de grasa en el hígado, en los riñones y en la sangre mostraron que un contenido de biotina de 120 µg/kg de comida parece satisfacer le necesidad mínima. Se recomiendan 160 µg/kg de comida, pues constituye el nivel que prevenía la dermatitis, muertes relacionadas con el síndrome de la hipertrofía del hígado y de los riñones así como deformaciones de los pies.