Biotin studies in pigs

1. Biotin deficiency in the young pig

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Sixteen pigs were given a purified diet of maize flour and casein from 5 to 25 d of age with or without a biotin supplement (100 µg/kg). From 26 to 94 d of age, eight of the pigs were changed to a wheat and casein diet with or without the biotin supplement and eight continued on the maize flour and casein diet. The weight gain and feed conversion ratio of the pigs were not affected by the dietary biotin supplement or the carbohydrate source. The pigs given the unsupplemented maize flour diet developed foot lesions, scaly skins and showed a reduction in carcass length. The excretion of biotin in the faeces was similar for the pigs given the biotin-supplemented and unsupplemented diets. The excretion of biotin in the faeces of pigs given the wheat diet was ten times as great as that of the pigs given the maize flour diet. The excretion of biotin in urine was 6 µg/d in the pigs given the unsupplemented maize flour diet and 67 µg/d in the pigs given the biotin-supplemented diet between 91 and 94 d of age. The biotin contents of the liver, heart, kidney, adrenals and plasma were increased by biotin supplementation of the maize flour diet. The 16:1/16:0 and 18:1/18:0 fatty acids ratios in the liver were decreased by biotin supplementation of the maize flour diet.

Biotin deficiency: Pig

Biotin deficiency was first induced in pigs by feeding a semi-purified diet containing egg-white (Cunha *et al*. 1946). Egg-white contains the glycoprotein avidin, which binds biotin making it unavailable to the animal. Later, Lehrer *et al*. (1952) also induced biotin deficiency in pigs by feeding a milk diet devoid of biotin. After this early work, it was thought that, since biotin was widely distributed in feedstuffs and was abundantly synthesized by the intestinal microflora, and since biotin deficiency was difficult to induce, the supply of the vitamin to the pig was usually sufficient to meet requirements. However, the report of a biotin-responsive condition in sows (Brooks *et al*. 1977) demonstrated that the supply of biotin is not always adequate and suggested that the biotin requirement of pigs should be evaluated.

The most recent National Research Council (1988) and Agricultural Research Council (1981) reports on the nutrient requirements of pigs both suggest a need for biotin by the pig, but only give estimated values as no requirements have been established. In the present experiment, an attempt was made to induce biotin deficiency in young pigs without the inclusion of avidin in the diet. In addition, a number of variables were measured to determine suitable criteria of response for the establishment of the biotin requirement of pigs.

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Table 1. *Composition of diets (g/kg)*

Ingredient	Diet ...	1	2	3	4	5
Casein		284·2	232·7	186·2	96·2	39·4
Maize flour		267·5	674·1	729·2	—	—
Wheat		—	—	—	858·6	923·0
Lactose		268·8	—	—	—	—
Maize oil		—	20·0	20·0	—	—
Calcium stearate		20·0	20·0	20·0	—	—
Calcium dihydrogen phosphate		37·8	32·4	27·0	32·4	27·0
Calcium carbonate		5·77	5·68	3·00	5·68	3·00
Potassium chloride		5·72	4·96	4·39	—	—
Sodium chloride		1·97	2·05	2·14	2·05	2·14
Magnesium sulphate		4·05	4·05	4·05	—	—
Trace mineral premix*		1·24	1·24	1·24	1·24	1·24
Vitamin premix†		2·73	2·73	2·73	2·73	2·73
Dried full-cream milk powder		100·00	—	—	—	—
Lysine		—	—	—	1·0	1·5
2,6-Di-*tert* butyl-*p*-cresol		0·050	0·025	0·025	0·025	0·025
Oxytetracycline quaternary salt		0·100	—	—	—	—

* Trace minerals (mg/kg diet): $FeSO_4 . 7H_2O$ 746·3, $ZnSO_4 . 7H_2O$ 440·53, $MnSO_4 . H_2O$ 30·8, $CuSO_4 . 5H_2O$ 23·7, KI 0·184, Na_2SeO_3 0·329.

† Vitamins (mg/kg diet): retinol 1·5, cholecalciferol 0·025, α-tocopherol 13·2, menadione 2·4, riboflavin 3·6, niacin 26·4, pantothenic acid 15·6, cyanocobalamin 26·4 μg, choline 1320, pyridoxine 1·8, folic acid 0·72, thiamin 1·56.

MATERIALS AND METHODS
Animals and diets

Sixteen entire male Landrace–Large White pigs (2 kg initial live weight) were weaned at 2 d of age. The pigs were housed in two groups for a preliminary period of 3 d and fed on diet 1 (Table 1) mixed with milk powder until they were eating the solid diet independently. At 5 d of age they were allocated, by restricted randomization on the basis of initial live weight, to two groups of eight pigs and given diet 1 based on maize flour and casein (Table 1) with or without a biotin supplement of 100 μg biotin/kg diet. At 26 d of age, the two groups of eight pigs were further subdivided, by restricted randomization on the basis of live weight. All pigs remained on the same supplemental biotin level: eight pigs (four from each biotin level) continued on a diet with maize flour, diet 2 (Table 1), and the remaining eight pigs were given a diet with wheat, diet 4 (Table 1), in a 2 × 2 factorial design. At 47 d of age, the pigs given diet 2 were given diet 3 and the pigs given wheat (diet 4) were given diet 5 until the end of the experiment (Table 1). During the experiment the pigs were fed *ad lib.* until 92 d of age and then were restricted by hourly feeding to 94 d of age (Kopinski *et al.* 1989). Fresh feed was offered daily, with pig weights and feed intakes measured weekly. Water was available *ad lib.* from nipple drinkers.

Pigs were housed individually in cages in a draught-free room maintained at 30° initially, reduced to 26° after 21 d. At 47 d of age the animals given the diet containing maize flour were transferred to metabolism crates, whereas the animals given the diet containing wheat were transferred to individual pens with partially slatted floors which were hosed clean twice daily to reduce coprophagy.

Collection procedure

Faeces and urine were collected between 23 and 26, 44 and 47, and 91 and 94 d of age. Faeces were collected on wire mesh over sloping trays which collected the urine into

buckets containing 50–100 ml 5 M-hydrochloric acid. Faeces were removed twice daily for 3 d, frozen immediately at −20°, then bulked and dried at 95° for 24 h in a fan-forced oven. Urine was removed daily for 3 d, a subsample was taken from each day's collection and bulked for each collection period and frozen at −20° until analysed.

Blood was collected by vena puncture at 33, 63 and 91 d of age. The plasma was separated by centrifuging at 3000 *g* for 10 min, and frozen at −20° until analysed. Serum was also collected for clinical biochemical tests.

The severity of foot lesions was assessed on a scale of 0 (no lesions)–5 (very severe lesions) at 33, 54, 61 and 94 d of age.

At 94 d of age the eight pigs given the maize flour diet were killed exactly 15 min after an hourly feed. Tissue samples (Table 3) were collected and frozen immediately at −20° until analysed. The carcass length was measured.

Analytical methods

Dry matter was determined for feed and faeces samples dried in a forced-air oven at 95° for 24 h. Biotin was analysed in feed and faeces samples after hydrolysis in 1 M-sulphuric acid according to the method of Hood (1977). The biotin content of the feed was also measured microbiologically by Hoffmann–La Roche, Basle, Switzerland. Urine samples were filtered then concentrated 20-fold by drying overnight at 95° before biotin assay (Hood, 1977). Tissue and plasma samples were also hydrolysed and analysed for biotin. Blood was analysed for various biochemical variables (serum enzymes, albumin, protein and urea-nitrogen) with a clinical analyser (Technicon, Tarrytown, NY, USA).

Total lipids in the liver were separated into fractions by thin-layer chromatography on pre-coated plates (silica gel 60: Merck) using the solvent system chloroform–methanol–acetic acid–water (85:15:10:3 by vol.) (Fogerty *et al.* 1985). The fractions were scraped from the plate as follows: fraction A containing three components, fraction B a 'phosphatidyl choline' fraction, fraction C a 'phosphatidyl inositol' fraction, fraction D a 'phosphatidyl ethanolamine' fraction, fraction E a 'neutral lipids' fraction. After removal, the lipid fractions were extracted from the absorbent, transmethylated and the crude fatty acid methyl esters were analysed by gas–liquid chromatography (Fogerty *et al.* 1985).

Statistical analysis

The design of the experiment was a 2 × 2 factorial with 4 replicates. There were two sources of carbohydrate and two levels of biotin (Steel & Torrie, 1980).

RESULTS

The biotin contents of the unsupplemented diets were 10 μg/kg for the maize flour diets and 80 μg/kg for the wheat diet. There was no effect of supplemental biotin on the performance of pigs between 5 and 82 d of age when given either a maize flour or wheat diet (Table 2). However, in the later stages of the experiment one animal given the unsupplemented maize flour diet did have a poorer performance. This was attributed to a low feed intake due to difficulty in moving, standing and eating, all of which occurred in this animal, which also exhibited severe foot lesions.

Table 2 shows the development of foot lesions with time in pigs given diets either with or without biotin supplementation, as assessed visually using a scale from 0 (no lesions) to 5 (very severe lesions) (Kopinski *et al.* 1986). The most severe lesions occurred in animals given the maize flour diet without biotin supplementation. In these animals there were deep cracks in the hoof, necrosis along the coronet, extensive sloughing of the heel horn and the corium was hyperplastic.

Table 2. *The performance and biotin excretion of young pigs given diets 1–5 with and without supplementary biotin**

(Means values for four pigs)

Diet...	Maize flour (1, 2, 3)		Wheat (4, 5)		SEM	
					Biotin CHO	Inter- action
Biotin supplement (μg/kg)...	0	100	0	100		
Weight gain (g/d)						
5–47 d	335	350	370	370	11·4	16·1
47–82 d	596	659	737	648	52·7	74·5
Feed conversion ratio						
5–47 d	1·20	1·22	1·16	1·19	0·02	0·03
47–82 d	2·08	2·00	2·11	2·43	0·10	0·14
Foot lesion score†						
33 d	0	0	0	0	—	—
54 d	1·5	1·3	1·0	0·8	0·07	0·11
61 d	2·3	0·6	0·8	0·6	0·08	0·12
94 d	4·1	0·5	—	—	0·42	—
Biotin intake (μg/d)						
44–47 d	4·9	49·5	38	86·4	—	—
91–94 d	16	176	—	—	—	—
Biotin excretion in faeces (μg/d)						
23–26 d	8·9	13·8	—	—	1·61	—
44–47 d	22·3	12·0	244	321	23·5	33·2
91–94 d	96	114	—	—	23·0	—
Biotin excretion in urine (μg/d)						
44–47 d	25·1	43·7	51·8	58·5	5·74	8·12
91–94 d	6·2	67·2	—	—	1·52	—

CHO, carbohydrate.
* For details, see Table 1 and p. 752.
† Assessed on a scale from 0 (no lesions) to 5 (very severe lesions); see Kopinski *et al.* (1986).

The skin of the most deficient animals given the unsupplemented maize flour diet was dry and flaky; there was loss of hair on the hams, and a development of brownish encrustations and pustules on the skin. In addition, the most deficient animals also exhibited 'furry tongue', a white film on the surface of the tongue; this later thickened, and transverse grooves appeared. In contrast, pigs given the diets supplemented with biotin did not display any of these symptoms on the skin or tongue, the hoof horn was sound, with only a very few superficial blemishes, and the heels appeared normal and healthy.

The length of the carcass was greater in the pigs given the maize flour diet with a biotin supplement than in those given the unsupplemented diet. The dressed weight was similar for both groups (Table 3). Comparison of the backfat thickness in the loin, mid and shoulder regions suggested that the pigs given the unsupplemented diet had more backfat in the shoulder region and less in the loin region compared with the pigs given the diets supplemented with biotin (Table 3).

The biotin concentration in all tissues examined was lower in animals given the maize flour diets without the biotin supplement than in pigs given the biotin-supplemented diets (Table 3). The concentration of biotin in the plasma of the pigs given the unsupplemented maize flour diets was lower at all ages than the concentration of biotin in the plasma of the pigs given the biotin-supplemented maize flour diets. Pigs given the wheat diets had higher concentrations of biotin in the plasma than the pigs given the maize flour diets. At 63 d of

Table 3. *Carcass measurements and the concentration of biotin in various tissues of pigs given diets 1–5 with and without supplementary biotin**

(Mean values for four pigs)

Diet...	Maize flour (1, 2, 3)		Wheat (4, 5)		SEM Biotin CHO	SEM Inter-action
Biotin supplement (μg/kg)...	0	100	0	100		
Carcass						
Dressed weight (kg)	28·4	31·8	—	—	2·62	—
Carcass length (mm)	568	623	—	—	18·9	—
Backfat (mm)						
Loin	9·8	11·9	—	—	1·48	—
Mid	20·2	18·9	—	—	1·68	—
Shoulder	36·0	28·3	—	—	3·25	—
Biotin in tissues (ng/g)						
Liver	31	248	—	—	16·9	
Heart	78	131	—	—	7·9	—
Kidney	108	515	—	—	40·6	—
Adrenals	69	139	—	—	13·7	—
Biotin in plasma (ng/l)						
33 d of age	149†	687†	—	—	74·4	—
63 d of age	161	673	674	1204	83·9	118·6
91 d of age	636	1683	2580	2000	182·2	257·5

CHO, carbohydrate.
* For details, see Table 1 and p. 752.
† *n* 8.

age the pigs given the unsupplemented wheat diets had a significantly lower concentration of biotin in the plasma than pigs given the same diet supplemented with biotin. By 91 d of age there was no difference in the concentration of biotin in the plasma of pigs given the biotin-supplemented or the unsupplemented wheat diets.

Biotin supplementation of the maize flour diet resulted in lower activities of aspartate aminotransferase (*EC* 2.6.1.1), lactic dehydrogenase (*EC* 1.1.1.27) and alanine amino-transferase (*EC* 2.6.1.2) in the serum (Table 4).

Table 5 shows the fatty acid ratios in various liver lipid fractions. The 16:1/16:0 ratios in liver 'phosphatidyl choline' (fraction B), 'phosphatidyl ethanolamine' (fraction D) and 'neutral lipids' (fraction E), were higher in the pigs given the diet without biotin. Moreover, the 18:1/18:0 ratios in liver 'phosphatidyl inositol' (fraction C) and 'neutral lipids' (fraction E) were also higher in these pigs.

The excretion of biotin in faeces increased with increasing age of the pigs (Table 2). The supplementation of the diets with biotin did not change the faecal excretion of biotin. However, in the pigs given the diets containing wheat the excretion of biotin in faeces was ten times that in pigs given the diets containing maize flour.

At 44 d of age the pigs given the maize flour diet excreted more biotin in urine when the diet was supplemented with biotin, but urinary biotin excretion was not altered in pigs given the wheat diet when the diet was supplemented with biotin. By 91 d of age the daily excretion of biotin in urine had decreased in the pigs given the unsupplemented maize flour diet, but had increased in the pigs given the supplemented diet (Table 2).

Table 4. *Serum constituents in pigs given the maize flour diet with or without supplementary biotin**

(Mean values for four pigs)

Blood constituent		Biotin supplement (μg/kg)		
		0	100	SEM
Aspartate aminotransferase (EC 2.6.1.1)	U/l	59·5	29·3	10·45
Creatine kinase (EC 2.7.3.2)	U/l	845	779	109·7
Alkaline phosphatase (EC 3.1.3.1)	U/l	315	319	93·3
Lactate dehydrogenase (EC 1.1.1.27)	U/l	922	612	105·7
Alanine aminotransferase (EC 2.6.1.2)	U/l	48·3	24·3	4·55
Albumin	g/l	45·2	37·3	1·64
Total protein	g/l	66·0	59·8	1·51
Blood urea-nitrogen	mM	4·63	3·40	0·779

* For details, see Table 1 and p. 752.

DISCUSSION

In the present study, giving a semi-purified diet, essentially devoid of biotin, to early-weaned pigs resulted in the development of dermal and foot lesions characteristic of biotin deficiency (Whitehead *et al.* 1975; Kornegay, 1986). A number of reports (Cunha *et al.* 1946; Pohlenz, 1974; Glattli *et al.* 1975; Hamilton *et al.* 1982) have suggested that biotin is required for growth, but in the present study and in the work of Lehrer *et al.* (1952) and Newport (1981) biotin deficiency was not accompanied by a growth depression. This apparent contradiction would appear to be due to the use of egg-white as a source of avidin in the diets of Cunha *et al.* (1946) and other workers, as growth depression has only been noted when egg-white has been included in diets. Tuszynska (1969) and Schrijver *et al.* (1979) showed that biotin supplementation of diets containing egg-white did not result in rat growth equivalent to that observed in animals fed on a laboratory chow of much lower biotin content. This suggested that the growth depression observed when egg-white was given was not entirely due to biotin inadequacy.

Egg-white contains constituents other than avidin that could be involved in growth depression. These are the ovoinhibitor proteins and ovomucoids that have trypsin-inhibitor properties (Osuga & Feeney, 1977). In vitro studies by Zahnley (1974) showed that porcine trypsin is inhibited by ovoinhibitor. If such activity is present in vivo, then the inhibition of trypsin activity will have an effect in lowering digestibility, resulting in poor performance.

In the present experiment, many of the problems of using an egg-white-based diet were overcome by the use of a semi-purified diet based on maize flour and casein. This allowed *ad lib.* feeding to be used, and so both animal groups received sufficient exogeneous energy supply, so that any responses observed following biotin supplementation would be uncomplicated biotin responses only. In addition, the early weaning of the pigs onto the solid diets at 2 d of age would remove the influence of biotin in milk and the consumption of biotin in the faeces of sows, and would reduce the time required to deplete the animals' stores of biotin. The performance results obtained in the present experiment are in

Table 5. *Fatty acid ratios in liver lipid fractions of pigs given a maize flour diet with and without supplementary biotin**

(Mean values for four pigs)

Fatty acid ratio	Biotin supplement (μg/kg)	Lipid fraction†				
		A	B	C	D	E
16:1/16:0	0	0·120	0·108	0·128	0·268	0·225
	100	0·140	0·077	0·147	0·157	0·163
	SEM	0·015	0·005	0·019	0·025	0·013
18:1/18:0	0	0·495	0·820	0·885	0·345	1·735
	100	0·370	0·697	0·643	0·303	1·330
	SEM	0·033	0·079	0·034	0·026	0·093
20:4/18:2	0	—	1·055	0·833	1·538	0·913
	100	—	0·903	0·660	1·147	1·013
	SEM	—	0·184	0·235	0·104	0·049

* For details, see Table 1 and p. 752.
† For descriptions of lipid fractions A–E, see p. 753.

agreement with those of Lehrer *et al.* (1952) and Newport (1981) in that there appears to be no need to supplement diets with biotin for normal pig growth. However, the occurrence of classical biotin deficiency lesions on hoof, skin and tongue in pigs fed on the unsupplemented maize flour diet suggests a requirement for biotin in maintenance of hoof and skin integrity. In the present experiment, it was observed that if biotin deficiency was allowed to progress long enough a growth effect would become evident in severely biotin-deficient animals, as shown by the reluctance of one very deficient animal to stand, move or eat after a period of 3 months, which caused a depression in feed intake. This, combined with an inadequacy or reduced activation of biotin-related endogenous metabolic pathways used in maintaining energy homeostasis, would ultimately cause reduction in growth. Despite the normal weight gains of pigs fed on the unsupplemented diet, a decrease in carcass length and a redistribution of carcass fat deposition suggests that biotin is required for normal development.

The pigs fed on the unsupplemented maize flour diet showed lower concentrations of biotin in the main biotin storage organs, the liver and kidney. A total elimination of biotin from these organs was highly unlikely, with the probable presence of biotinidase (*EC* 3.5.1.12) activity allowing recycling of endogenous biotin. A functional biotinidase confers on the animal some independence from exogenous biotin supply, with conservation and recycling of intracellular biotin a number of times before it is lost from the cell and then the animal's body (Wolf *et al.* 1985).

In the laying hen, [^{14}C]biotin studies (White *et al.* 1976) have shown a half-life for biotin of 25 d. In humans it has been found that, even after 50 d of starvation, biotin excretion in the urine remained unchanged (Swendseid *et al.* 1965), suggesting an even longer half-life. Such long half-lives explain the difficulty of these studies and the long periods of biotin depletion necessary before biotin deficiency symptoms are observed.

The biotin excretion patterns in the pigs in the present experiment suggest that faecal biotin excretion is independent of biotin intake. Tagwerker (1978), quoting the unpublished results of L. Volker and co-workers, also reported that faecal biotin excretion was similar at all levels of biotin intake. The concentration of biotin in faeces is more a reflection of microbial biotin production in the hindgut than of undigested biotin from food residues (Kopinski *et al.* 1989). Radioactive studies on saliva in marmosets (*Saguinus fusicollis*)

(Dreizen & Hampton, 1969) and chicken bile (Frigg, 1978) have not demonstrated any significant contribution from these sources to faecal biotin levels. The increase in biotin excretion with age is related to the increase in feed intake, causing an increase in microbial biotin synthesis and increased total faecal output.

The higher excretion of biotin in the faeces of pigs given the wheat diets compared with those given the maize flour diet shows that dietary composition influences biotin excretion through alteration of microbial activity in the intestines. The occurrence of biotin deficiency symptoms in pigs fed on the unsupplemented diets suggests that such microbially synthesized biotin contributes little or nothing to the biotin status of the pig.

Excretion of biotin in urine, unlike that in faeces, more closely reflected the influence of biotin supplementation. The report of Lehrer et al. (1952) is the only work with pigs in which excretion of biotin in urine was compared in pigs given diets unsupplemented and supplemented with biotin. With increased biotin intake, there was an increase in biotin excretion in the urine. The excretion of biotin in the urine of the unsupplemented pigs was attributed to absorption and excretion of microbially synthesized biotin. However, in the light of recent knowledge on biotin recycling (Wolf et al. 1985) its continued excretion could be attributed to a slow partial turnover of the protein-bound biotin pool.

The concentration of biotin in the plasma of pigs fed on the unsupplemented maize flour diet reflected the depletion of biotin reserves in these pigs. The variability of plasma biotin levels between animals would prevent the use of plasma biotin as a technique for assessing the biotin status of individual animals, although the use of plasma biotin can reflect the biotin status of a group of pigs.

Three serum enzymes which are routinely analysed clinically seem to be modulated by the biotin status of the pigs. Normally, serum aspartate aminotransferase, lactate dehydrogenase and serum alanine aminotransferase activities, when increased, reflect either acute hepatic disease or myocardial infarction in animals. However, increases in the activities of two of these enzymes in the liver during biotin deficiency in chickens have previously been reported (Balnave & Jackson, 1974). The increases observed in these three enzymes may be a means by which the pig may be able to utilize the excess pyruvate which could not be used by the cell for gluconeogenesis, fatty acid synthesis or tissue respiration to provide α-ketoglutarate or NAD^+, or to replenish oxaloacetate for use in the citric acid cycle. In other words, these increases in activity are an effort to maintain normal synthetic processes and tissue respiration during biotin deficiency (Balnave & Jackson, 1974).

The observed changes in the liver 16:1/16:0 and 18:1/18:0 fatty acid ratios were primarily due to the elevated levels of 16:1 and 18:1 fatty acids and appear to be a good indicator of biotin status. Brooks et al. (1977) also found a higher monoene saturate ratio in the depot fat of growing pigs given a biotin-deficient diet. It has previously been observed in chickens with the biotin responsive disorder, fatty liver and kidney syndrome, that there is a marked increase in the proportion of palmitoleic acid (16:1) in liver lipid (Whitehead et al. 1975; Hood et al. 1976). The increase in the proportion of unsaturated fatty acids in liver and adipose tissue of deficient animals has the undesirable economic effect of making carcass fat softer.

In conclusion, the results obtained in the present experiment indicate that normal weight gains may be achieved in pigs given a maize starch and casein diet without supplemented biotin. It is possible that the biotin synthesized and absorbed from the hind gut is sufficient to meet this requirement of the pig. However, there appears to be a requirement for supplemental biotin for the prevention of biotin deficiency symptoms such as hoof and skin lesions, and a biotin supplement of 100 μg/kg diet appears to be adequate to prevent the occurrence of these symptoms.

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