Biotin studies in pigs

2. The biotin requirement of the growing pig

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Twenty pigs weaned at 5 d were given pelleted diets based on maize flour and casein and supplemented with 0, 50, 100, 150 or 200 μg biotin/kg diet. The performance of the pigs was not influenced by the biotin content of the diets. Typical biotin deficiency symptoms (foot lesions and skin pustules) were observed in pigs given the unsupplemented diet and the diet supplemented with 50 μg biotin/kg diet. Tissue biotin concentration plateaued when 100 μg biotin/kg was added to the diet. Faecal biotin excretion was independent of dietary biotin intake, but increased with age. Urine biotin excretion at 102 d was significantly lower for the unsupplemented pigs than for the pigs given various levels of dietary biotin supplements. A dietary requirement of biotin for young pigs between 50 and 100 μg/kg diet is suggested from the results of the present experiment.

Biotin requirement: Pig

The most recent National Research Council (1988) and Agricultural Research Council (1981) reports on nutrient requirements of pigs both indicate a need for biotin by the pig, but only give suggested values as no requirements have been established. The current suggested levels of biotin (50 and 100 μg biotin/kg diet) are based on early work by Cunha *et al.* (1946) who found that the injection of 100 μg biotin/d was sufficient to prevent the occurrence of biotin deficiency symptoms in pigs in which biotin deficiency had been induced by the inclusion of dried, raw egg-white in the diet.

More recent studies by Hamilton *et al.* (1983) suggested that 90 μg biotin/kg diet provided in a diet containing autoclaved egg-white was sufficient to meet the biotin requirement of weanling pigs. An attempt was made in the present experiment to determine the requirements for biotin in young pigs without the inclusion of egg-white as a source of avidin in the diet, by employing a semi-purified diet which had previously been used to induce biotin deficiency (Kopinski *et al.* 1989).

MATERIALS AND METHODS

Animals and diets

Twenty entire male Landrace–Large White pigs (1·5 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 given diet 1 (Table 1). At 5 d of age they were allocated, by restricted randomization on the basis of live weight, to five groups of four pigs and given diet 1 (Table 1) supplemented with 0, 50, 100, 150, or 200 μg biotin/kg diet. After 30 d of age the pigs were given diet 2 (Table 1), while being maintained on their respective biotin supplements. After 68 d of age the pigs were given diet 3 with their respective biotin supplements until the end of the experiment.

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

Ingredient	Diet...	1	2	3
Casein		284·2	232·7	186·2
Maize flour		366·6	674·1	729·2
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
Calcium carbonate		5·77	5·68	3·00
Potassium chloride		5·72	4·96	4·39
Sodium chloride		1·97	2·05	2·14
Magnesium sulphate		4·05	4·05	4·05
Trace minerals premix*		1·24	1·24	1·24
Vitamin premix†		2·73	2·73	2·73
2,6-Di-*tert* butyl-*p*-cresol		0·050	0·025	0·025
Lecithin		1·00	—	—
Oxytetracycline quaternary salt		0·10	—	—

* 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.

Throughout the experiment the pigs were fed *ad lib.*, with fresh feed offered daily; water was always available *ad lib.* from nipple drinkers. Pig weights and feed intakes were measured weekly.

Pigs were kept in a draught-free room maintained at 30° initially, reduced to 26° after 21 d. The pigs were housed in individual cages.

Collection procedure

Faeces were collected on wire mesh over sloping trays which collected the urine into buckets containing 1 M-sulphuric acid for 3 d between 13 and 15, 27 and 29, 69 and 71, and 101 and 103 d of age. 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 for each collection day and bulked into a collection-period sample and frozen at −20° until analysed.

Blood was collected by vena puncture at 47 and 68 d of age. Plasma was separated by centrifuging at 3000 ***g*** for 10 min, and frozen at −20° until analysed. At 35 and 102 d of age, hair samples were collected by shaving a patch of skin on the dorsal surface of the pigs, the same site of sampling was used for all the pigs. At the conclusion of the experiment at 103 d of age, various tissue samples were collected from the animals after slaughtering. The severity of foot lesions was assessed on a scale of 0 (no lesions) to 5 (very severe lesions) at 94 d of age (Kopinski *et al.* 1986).

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 on feed, faeces, tissue and plasma samples after hydrolysis in 1 M-H_2SO_4 according to the method of Hood (1977). Urine samples were filtered then concentrated 20-fold by drying overnight at 95° before biotin assay (Hood, 1977).

Statistical analysis

All observations were subjected to one-way analysis of variance (Steel & Torrie, 1980).

Table 2. *The performance of pigs given semi-purified diets supplemented with biotin*, and the occurrence of foot lesions*

(Mean values for four pigs)

Biotin supplement (μg/kg diet)...	0	50	100	150	200	SEM
Wt gain (g/d)						
8–50 d	208	208	218	223	226	11·8
50–80 d	602	601	601	589	608	12·4
Feed conversion ratio						
8–50 d	1·07	1·09	1·11	1·10	1·07	0·016
50–80 d	1·23	1·33	1·30	1·34	1·34	0·040
Foot lesion score†	4·0	1·2	0·5	0·5	0·5	0·21

* For details, see Table 1 and p. 761.

† Assessed on a scale from 0 (no obvious lesions) to 5 (very severe lesions); see Kopinski *et al.* (1986).

Table 3. *The biotin content of tissues and hair (ng/g) in pigs given semi-purified diets supplemented with biotin**

(Mean values for four pigs)

Biotin supplement (μg/kg diet)...	0	50	100	150	200	SEM
Liver	220	651	589	585	800	115
Kidney	358	827	1146	1170	1095	83
Heart	20	63	62	67	75	3·0
Hair						
35 d	4	11	5	11	10	4·3
102 d	20	38	73	71	81	23·7

* For details, see Table 1 and p. 761.

RESULTS

The weight gains and feed conversion ratios for the pigs (Table 2) showed no differences between levels of dietary biotin supplementation of 0–200 μg/kg. The basal diet contained 10 μg biotin/kg.

The foot lesions (Table 2) at various ages showed that the pigs given the unsupplemented diet had significantly higher foot lesion scores, as assessed visually, than pigs fed on the higher supplements of biotin. Pigs supplemented with 50 μg biotin/kg diet also exhibited some foot lesions. However, the severity of these lesions was much less than for the pigs fed on the supplemented diet. In the most severely deficient animal the lesions on the hoof were most pronounced, with heel horn sloughing, a hyperplastic corium and necrosis of the coronet with some very deep cracks in the hoof. Some of the deficient animals exhibited dry flaky skin; there was hair loss on the hams, and brownish encrustation and pustule formation on the skin. Pigs given diets supplemented with more than 50 μg biotin/kg diet did not display any of these symptoms, with hoof horn being sound and only occasional superficial blemishes on the skin. Tongue and heels appeared normal and healthy in these pigs. In the unsupplemented pigs the tongue was covered with a white scale.

The liver and kidney are important storage organs for biotin, with concentrations of biotin ten times those found in the heart (Table 3). Pigs given the unsupplemented diet had significantly lower concentrations of biotin in all their tissues than pigs fed on the diets supplemented with biotin (Table 3). The findings also show that there were no further

Table 4. *Faecal and urinary excretion of biotin (μg/d) and biotin in blood plasma (ng/l) in pigs given semi-purified diets supplemented with biotin**

(Mean values for four pigs)

Biotin supplement (μg/kg diet)...	0	50	100	150	200	SEM
Faeces						
14 d	2·2	2·3	1·5	1·7	1·0	0·83
28 d	2·8	2·0	3·8	3·9	3·3	0·91
70 d	15·1	22·2	18·0	21·4	19·5	4·58
102 d	39·8	32·2	30·1	28·4	33·8	6·93
Urine						
14 d	2·4	†	†	†	†	†
40 d	8·3	†	†	†	†	†
70 d	23·6	†	†	†	†	†
102 d	25·0	55·0	53·0	68·0	69·0	9·4
Plasma						
47 d	70	140	230	540	600	125
68 d	880	1000	950	1010	1470	127

* For details, see Table 1 and p. 761.
† Not collected.

increases in the concentration of biotin in tissues with additional biotin supplementation above 100 μg biotin/kg diet. Analysis of hair (Table 3) indicated that the biotin content is significantly higher at 102 d of age than at 35 d of age, and that—at least at 102 d of age—it increased with biotin supplementation to 100 μg/kg diet.

Examination of the faecal excretion of biotin throughout the experiment (Table 4) showed that faecal biotin excretion increased with age, and that the amount excreted at each age period was not significantly different between the various levels of biotin supplementation. It was also found that the biotin concentration of faeces over all age-periods and biotin supplements was relatively constant at about 450 ng/g faeces.

During the early portion of the experiment urine was collected only from the pigs given the unsupplemented diets. Table 4 indicates that with increasing age there was an increase in the urinary excretion of biotin. At 102 d of age the excretion of biotin was significantly lower in the pigs given the unsupplemented diet than in the pigs given the biotin-supplemented diets.

At 47 d of age the concentrations of biotin in the plasma appeared to increase with increasing level of biotin supplementation (Table 4), but at 68 d of age the differences were small.

DISCUSSION

The lack of an established nutrient requirement for biotin in pigs (National Research Council 1988; Agricultural Research Council, 1981) may be due to the wide distribution of biotin in feedstuffs and the abundant synthesis of biotin by the intestinal microflora. Biotin-responsive conditions in sows have been reported recently by Brooks *et al.* (1977). Before discussing reports on the biotin requirements of pigs, it is necessary to partition the biotin requirement into two: the biotin requirement for growth, and the biotin requirement for the prevention of the development of biotin deficiency symptoms. The need for such a separation becomes evident from the results obtained in the experiments of Lehrer *et al.*

(1952) and our previous work (Kopinski *et al.* 1989), where symptoms of biotin deficiency were produced in pigs, but where no growth response was observed following biotin supplementation of the biotin-deficient diets. These findings indicate that the requirement for growth is lower than the requirement for preventing the occurrence of biotin-deficiency symptoms.

The first report on the production of biotin deficiency in the pig (Cunha *et al.* 1946) proposed a nutrient requirement for biotin of 100 μg biotin/d. This was the amount of biotin injected into the control groups of pigs to prevent their becoming biotin deficient when given a diet containing dried, raw egg-white. In a more recent study (Hamilton *et al.* 1983) it was suggested that 90 μg biotin/kg diet met the pigs' requirement for biotin. This was true for the prevention of biotin-deficiency lesions occurring in that experiment. However, in the light of the questionable validity of findings from studies using diets based on egg-white in evaluating the influence of biotin on growth, the value of 90 μg biotin/kg diet may not be an accurate estimate of biotin requirement for pig growth. The experiment of Hamilton *et al.* (1983) failed to include an unautoclaved egg-white diet given to the group of pigs supplemented with biotin. If such a treatment group had been included in the experiment and had shown normal growth with no visible deficiency lesions, then it could have been concluded that the biotin supplement provided did meet the requirements for growth and prevention of deficiency symptoms. However, the improvement in growth observed when pigs were fed on an autoclaved egg-white diet cannot be attributed to avidin inactivation and the presence of 90 μg biotin/kg diet only. Autoclaving also inactivates other egg-white proteins, in particular ovoinhibitor and ovomucoid, both of which are trypsin inhibitors (Osuga & Feeney, 1977). Porcine trypsin has been shown to be inhibited by ovoinhibitor in vitro (Zahnley, 1974). If such activity were functional in vivo, then pigs fed on the untreated egg-white diet would have lower digestibilities and poorer performance, whereas animals fed on the autoclaved egg-white diet with such activity destroyed would have higher digestibilities and better performance. Such a response was observed in the report of Hamilton *et al.* (1983), with their plasma urea values confirming the effect on digestibility. Unfortunately these responses were mistakenly identified as biotin responses and not trypsin-inhibitor inactivation responses.

The performance of the pigs in the present experiment confirms our earlier results (Kopinski *et al.* 1989), and is in agreement with those of Lehrer *et al.* (1952) and Newport (1981) in that there is no requirement for supplemental biotin for the growth of pigs. The average weight gain of similar pigs under farm conditions is 300 g/d from 8 to 50 d and 520 g/d from 50 to 80 d. Thus, the weight gains from 8–50 d were lower in the present experiment (208–226 g/d) than under farm conditions or in the experiment of Kopinski *et al.* (1989) where the dietary essential fatty acids were provided by dried full-cream milk. However, in the latter stages of growth the weight gains of the experimental pigs were greater than those experienced under farm conditions. In pigs given the unsupplemented diet, classical biotin deficiency lesions on hoof and skin were observed to develop. The animals given the diet supplemented with 50 μg biotin/kg exhibited some foot lesions; however, they were less frequent and less severe than those found in the pigs given the unsupplemented diet. This suggests that a supplement of 50 μg biotin/kg diet was only marginal as there was a total absence of lesions with only occasional superficial blemishes on the hooves of pigs given the diet supplemented with 100 μg biotin/kg.

The pigs given the unsupplemented diet had significantly lower biotin concentrations in the liver, kidney and heart. Overall, the tissue biotin concentrations in the present experiment are much higher that those observed in earlier work (Kopinski *et al.* 1989); however, this may be attributable to the source of the piglets used in the present experiment. The source of pigs was the same in both experiments, except that between the

initial experiment (Kopinski *et al.* 1989) and the present experiment the piggery commenced supplementation of the whole herd with biotin, so that the piglets used in the second experiment probably contained higher endogenous levels of biotin in the tissues at birth. A functional biotinidase (*EC* 3.5.1.12) would allow some conservation and recycling of this intracellular biotin and a significant residue of biotin in the tissues even after a long depletion period.

The concentrations of biotin in the tissues and hair reached plateaux with a supplement of 100 μg biotin/kg diet. This would seem to suggest a saturation of biotin-binding sites in these tissues, this saturation indicating that the requirement for biotin by the pig was met with 100 μg biotin/kg diet.

As in the previous study (Kopinski *et al.* 1989), faecal biotin excretion was independent of biotin intake. The pattern of faecal biotin excretion indicates that the increased biotin excretion with age was entirely due to increased microbial synthesis of biotin and increased faecal output. The presence of biotin deficiency symptoms in pigs fed on the unsupplemented diet indicates that the pigs derive only limited benefit from this microbiologically synthesized biotin in the hind gut when coprophagy was prevented. However, the backflow of this biotin into the intestines and its subsequent absorption may have provided sufficient biotin for growth in these pigs.

In conclusion, the present study has confirmed our earlier results in that there is no requirement for supplemental biotin for weight gain; however, the biotin required in the diet for prevention of hoof lesions appears to be between 50 and 100 μg/kg diet. This amount of biotin also prevented skin lesions, furry tongues and loss of hair and resulted in higher concentrations of biotin in tissues and hair compared with that found in the unsupplemented pigs.

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