Biotin studies in pigs

3. Biotin absorption and synthesis

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Eight pigs were given a semi-purified diet based on maize flour and casein containing 10 μg biotin/kg. The diet was given *ad lib.* with or without a supplement of 70 μg biotin/kg diet from 5 to 94 d of age. The flow of biotin in the stomach was similar to the biotin intake (13·5 and 112 μg/d) for the unsupplemented and biotin-supplemented pigs respectively. The flow of biotin through the small intestine decreased for the biotin-supplemented pigs from 39 μg/d in the first quarter of the small intestine to 7·9 μg/d in the last quarter. The flows of biotin in the caecum, large intestine and colon were similar for both the unsupplemented and biotin-supplemented pigs, with values of 17–54 μg/d, indicating the synthesis of biotin in the hind-gut.

Biotin synthesis: Pig

Few studies have been carried out on the site of absorption of vitamins in the pig. However, studies with vitamin B_{12} (Holdsworth & Coates, 1961) showed that the central part of the small intestine was the main region for absorption. Evidence of colonic uptake of vitamin B_{12} was presented by Henderickx *et al.* (1964). More recently Ford *et al.* (1975) showed that piglet mucosa in the proximal and central region of the small intestine took up proteins which bound vitamin B_{12} and folic acid.

Biotin administered orally to pigs was found to be absorbed rapidly, as shown by a marked increase in the concentration of biotin in the plasma within 1 h of feeding (H. R. Glattli, unpublished results quoted by Tagwerker, 1978). Volker & Smith (1980) found that there was very little absorption of [^{3}H]biotin from the caecum of pigs. Barth *et al.* (1986) found that 18% of the biotin infused into the caecum was excreted in urine. This limited information would suggest that the major site of biotin absorption is the upper intestinal tract, that is, the region where most nutrient absorption occurs in the pig.

The present experiment was designed to locate the sites and quantify the amount of both absorption and synthesis of biotin in the young pig. The information obtained would be useful in allowing determination of the contribution of biotin to the pig from feed ingredients and the direct contribution if any to the biotin status of pigs of microbial biotin synthesis in their gastrointestinal tracts.

MATERIALS AND METHODS

Animals and diets

Eight entire male Landrace–Large White pigs were given a maize flour and casein diet (diet 1, Table 1) supplemented with 0 or 70 μg biotin/kg diet after weaning at 2 d (Kopinski

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

Diets...	1	2	3
Casein	284·2	232·7	186·2
Maize flour	267·5	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 mineral premix*	1·24	1·24	1·24
Vitamin premix†	2·73	2·73	2·73
Dried full-cream milk powder	100·00	—	—
2,6-Di-*tert* butyl-*p*-cresol	0·050	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.

et al. 1989). Diet 1 was given to pigs to 25 d of age, diet 2 from 26 to 46 d of age and diet 3 from 47 to 94 d of age. The pigs were offered fresh feed daily *ad lib.* and were housed in metabolism cages which prevented access to faeces.

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

Indigestible markers CrEDTA (280 mg chromium/kg diet) and ytterbium nitrate (160 mg ytterbium/kg diet) were sprayed on diet 3 given to the pigs from 89 to 94 d of age. From day 92, feed was restricted to hourly feeds of 80 g/pig. On day 94, each pig was killed exactly 15 min after an hourly feed by captive bolt stunning followed by jugular rupture. The intestinal tract was rapidly removed and the length of the small intestine was measured. The small intestine was then divided into four parts of equal length. Similarly the large intestine was measured, then divided into two parts of equal length. Digesta were collected quickly to prevent the shedding of epithelium into the intestinal lumen. Digesta were frozen immediately at −20° until analysed.

Analytical methods

Dry matter (DM) was determined for feed, faeces and digesta samples dried in a forced-air oven at 95° for 24 h. Analyses of Cr and Yb contents were carried out on dry samples digested in concentrated perchloric–nitric acids (1:3 v/v) and measured using an atomic absorption spectrophotometer (Varian Pty Ltd, Melbourne). Biotin was analysed in dry samples of feed, faeces and digesta after hydrolysis in 1 M-sulphuric acid by the method of Hood (1977). The flow of digesta in each portion of the digestive tract was calculated from the mean concentration of indigestible marker in each section.

Statistical analysis

Biotin concentration and flow, and DM flow were subjected to one way analysis of variance (Steel & Torrie, 1980).

Table 2. *The flow of dry matter (g/d), in the digestive tract of pigs given a maize flour and casein diet with or without supplementary biotin**

(Mean values for four pigs)

Dietary biotin supplement (μg/kg)...	0	70	
Segment			SEM
Feed	1364	1626	110·5
Stomach	1566	1670	61·4
SI 1	1066	1161	132·7
SI 2	472	417	88·8
SI 3	201	252	25·4
SI 4	146	147	23·1
Caecum	118	117	12·3
LI 1	103	100	8·9
LI 2	88	97	8·0
Colon	93	94	10·8
Faeces	97	97	8·9

SI, small intestine; LI, large intestine.
* For details, see Table 1 and p. 768.

RESULTS

All pigs given the unsupplemented diets developed foot lesions and pustules on the skin. The ratio of the mean retention time of Cr (as CrEDTA) to Yb was between 0·94 and 1·08 for all sections of the gastrointestinal tract except the stomach, where the ratio was between 0·70 and 0·80. This suggests that the calculated flow rates of digesta were equally valid from either marker for all sections except the stomach, where the flow was related to the retention of Yb.

The flow of DM in various portions of the intestinal tract of pigs given diets with and without biotin supplements is shown in Table 2. A similar pattern was observed for the flow of DM in the pigs given the biotin-supplemented or the unsupplemented diets. In each quarter of the small intestine the flow of DM decreased by about 50% from the level in the previous segment. By segment 4, DM flow was only 10% of intake. Less than 4% of the total absorption of DM occurred in the caecum, large intestine and colon.

In the pigs given the unsupplemented diet, there was a decrease in the concentration of biotin from the 10 μg biotin/kg in the diet to 3·3 μg/kg digesta DM in the second segment of the small intestine (Table 3). From the third segment of the small intestine the concentration of biotin increased gradually, the magnitude of the biotin concentration increase was from threefold the original feed content in the third segment of the small intestine to fifty-nine-fold in the second segment of the large intestine. In the pigs given the biotin-supplemented diet a similar trend was observed, with an initial decrease in biotin concentration from 80·5 μg biotin/kg diet to 24·1 μg biotin/kg digesta DM in the second segment of the small intestine. Again, the concentration of biotin in the various segments of the intestine increased gradually from the third segment of the small intestine to a concentration of 551 μg biotin/kg digesta in the colon. The biotin concentrations of digesta in the various post-ileal intestinal segments were similar for both diets.

The flow of biotin in pigs given the unsupplemented diet decreased 50% from the stomach (13·5 μg/d) to the small intestine segment 1 (6·1 μg/d). There was a further decrease in the flow of biotin in the small intestine segment 2 to 1·4 μg/d. In the small intestine segments 3 and 4, the flow was about 5·5 μg/d. In the caecum, the flow of biotin

Table 3. *The concentration and flow of biotin in the digestive tract of pigs given a maize flour and casein diet with or without supplementary biotin**

(Mean values for four pigs)

Segment	Dietary biotin supplement (μg/kg)...	Biotin concentration (ng/g DM)			Biotin flow (μg/d)		
		0	70	SEM	0	70	SEM
Feed		10·3	80·5	—	14·0	131	—
Stomach		8·3	68·3	5·26	13·5	112	5·06
SI 1		4·6	29·8	4·22	6·1	39·0	6·27
SI 2		3·3	24·1	11·26	1·4	9·9	5·24
SI 3		30·8	56·8	22·68	5·8	13·2	4·86
SI 4		44·8	55·8	16·37	5·1	7·9	1·96
Caecum		213	148	45·2	25·0	17·1	6·12
LI 1		371	369	68·0	38·6	35·5	7·09
LI 2		613	490	76·9	53·5	48·0	6·95
Colon		539	551	136·3	52·8	50·0	14·58

DM, dry matter; SI, small intestine, LI, large intestine.
* For details, see Table 1 and p. 768.

was twice the original intake (25 μg/d) and four times the original intake in the colon (53 μg/d). In the pigs given the biotin-supplemented diet, the flow of biotin declined slightly in the stomach (112 μg/d) from the feed intake (131 μg/d). However, the major decrease in flow or absorption occurred in the small intestine segment 1 (to 39·0 μg/d) and in the small intestine segment 2 (to 9·9 μg/d). In the small intestine segments 3 and 4 biotin flow was about 11·5 μg/d. As previously observed in the unsupplemented pigs, the flow of biotin increased in the caecum and large intestine.

DISCUSSION

Feed and intestinal microbial synthesis of biotin are often thought to provide adequate biotin to prevent deficiency symptoms in pigs. However, no information has been published to show that microbially synthesized biotin does contribute to the biotin status. Moreover, studies with chickens (Frigg, 1976) suggest that although some feedstuffs are high in biotin, the availability of such biotin is often low. The present experiment was designed to qualify and quantify the site and amount of biotin absorption and synthesis in the young pig.

A semi-purified diet with a low biotin content (10 μg/kg DM) and the same diet supplemented with 70 μg synthetic biotin/kg were given to pigs to evaluate the sites of biotin absorption. The experiments also allowed an estimation of microbial synthesis of biotin in various portions of the intestinal tract.

The decrease in the concentration of biotin in the small intestines of both groups of pigs indicated that biotin absorption was primarily occurring in the first half of the small intestine. The sites of biotin absorption were similar to the sites of absorption of DM. This is in agreement with in vitro studies of biotin absorption in various other animals (Spencer & Brody, 1964; Berger *et al.* 1972). However, as the biotin provided to the supplemented pigs in the present experiment was a free crystalline biotin, readily available for absorption, the lack of apparent absorption in the third and fourth segments of the small intestine does not indicate that absorption does not occur in these regions, only that the very available biotin supplied in the supplemented diets was absorbed before reaching the lower small intestine. With a more practical pig diet based on cereals, the second half of the small

intestine may play an equally or even more important role in the absorption of biotin, especially where the availability of biotin is low (Frigg, 1976).

The increase in the concentration of biotin in the second half of the small intestine could be due either to microbial synthesis of biotin within this section of the gut, or to backflow of digesta from the caecum. In the caecum and large intestine a high concentration of biotin was observed in pigs given both the unsupplemented and biotin-supplemented diets. There are two possible sources of this biotin, net secretion of biotin into these segments and microbial synthesis of biotin in these portions of the intestinal tract. The first possible cause, i.e. net secretion, is unlikely as radioactive studies on saliva in marmosets (*Saguinus fusicollis*) (Dreizen & Hampton, 1969) and chicken bile (Frigg, 1976) have not demonstrated biotin contribution to digesta from these fluids. Moreover, the pigs had been depleted of biotin from 2 d of age and this has been shown to result in low concentrations of biotin in tissues (Kopinski *et al.* 1989). The probable cause of the increase in biotin concentration in the hind-gut is the post-ileal microbial synthesis of biotin. It is well known that the microbial populations of the post-ileal segments of pigs are very large (Vervaeke *et al.* 1979) and that these are the primary regions of fermentation and vitamin synthesis in the intestines of most simple-stomached animals (Christensen, 1980). Moreover, many micro-organisms are known to be capable of biotin synthesis (McCormick & Wright, 1971), so that the observed increase in the concentration of biotin in those portions of the intestinal tract where fermentation occurs indicates microbial biotin production. The similar increases in the concentration of biotin in digesta in the pigs given both the unsupplemented and biotin-supplemented diets indicate that the biotin synthesis is independent of the biotin intake of the pig. The difference in the concentration of biotin in the caecum compared with the large intestine may be due to the variation in the composition or total numbers of the microbes in these segments.

With the technique of serial slaughter utilized in the present experiment, the presence of high concentrations of biotin in the post-ileal digesta from microbial synthesis means that biotin absorption, if occurring in the post-ileal intestinal tract, cannot be determined without radioactively-labelled biotin to distinguish the source of biotin detected in plasma and tissues. Previous research suggests that biotin transport or absorption is specific for D-biotin and only a few other biotin analogues (Spencer & Brody, 1964). The form of biotin in the hind-gut has not been determined.

In conclusion, even with extensive microbial synthesis of biotin in the post-ileal tract, the previously reported low concentrations of biotin in plasma and tissues and the presence of deficiency symptoms (Kopinski *et al.* 1989) indicate that post-ileally synthesized biotin is of insufficient benefit to the pig. The primary absorption site for crystalline biotin added to the diet was the first half of the small intestine. Although there was no apparent absorption of biotin in the second half of the small intestines in the pigs given the unsupplemented diets, there may have been some true absorption of biotin in this area from the backflow of biotin synthesized by the microbes in the lower digestive tract. Further experiments (Kopinski *et al.* 1989) with the use of [^{14}C]biotin will report on the quantitative post-ileal absorption of biotin.

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