The vitamin B_6 status of pigs given a diet containing linseed meal

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1. Pigs consuming a diet containing 300 g linseed meal/kg and a pyridoxine supplement showed greater growth, nitrogen retention, blood packed cell volume and haemoglobin than those receiving only the basal diet.

2. Tryptophan-load tests on unsupplemented pigs revealed an increased excretion of kynurenine, N^{α}-acetylkynurenine and xanthurenic acid compared to those receiving additional pyridoxine.

3. The results suggest that the unsupplemented pigs were marginally deficient in vitamin B_6.

4. When the same diet was fed to rats there was no evidence of vitamin B_6 deficiency.

Vitamin B_6 deficiency in pigs, which gives rise to a loss of appetite, poor growth, nervous disorders and anaemia, has been produced under experimental conditions by Chick, Macrae, Martin & Martin (1938) and Wintrobe, Follis, Miller, Stein, Alcayaga, Humphreys, Suksta & Cartwright (1943), but it is not known to occur under normal farm conditions. However, Kratzer (1946) found that the nutritive value of linseed meal for the chick was unsatisfactory unless the meal had previously been washed with water and dried. Subsequently, Kratzer & Williams (1948) found that linseed meal could be given to chicks without deleterious effects if either yeast extract or pyridoxine was added. An analogous situation was found by Tjostem, Diner, Parsons & Klosterman (1963) when rats received a diet containing linseed meal: poor growth and deaths resulted but both could be prevented by additional pyridoxine.

A vitamin B_6 antagonist was isolated from linseed meal by Klostermann, Lamoureux & Parsons (1967) and was identified as the dipeptide linatine, 1-(*N*-γ-L-glutamyl) amino)-D-proline. When linatine was hydrolysed, 1-amino-D-proline and glutamic acid were produced; the former was found to combine with pyridoxal phosphate in vitro, and when injected into chicks it was found to have greater toxicity than linatine.

In experiments reported by Bethke, Bohstedt, Sassaman, Kennard & Edington (1928) diets containing 250 g linseed meal/kg were fed to rats and pigs without adverse effect. In other experiments in which linseed oil meal was fed to pigs (Crampton, MacKay & Lloyd, 1955; Wahlstrom, Kamstra Olson, 1956; Taxilin, 1961; Jusyczak & Zieminski, 1968) lower levels were given than were used by Bethke *et al.* (1928) and no deleterious effects were attributed to the linseed meal; none of these trials were concerned with the vitamin B_6 status of the pigs. In the present paper,

Table 1. *Composition (g/kg) and chemical analysis (g/kg dry matter (DM)) of the linseed meal diet given to pigs and rats*

Composition	
Ground linseed meal	300·0
Ground barley	360·0
Ground oats	313·4
Vitamin–mineral supplement*	1·1
L-lysine	1·1
L-methionine	0·9
Calcium carbonate	10·0
Dicalcium phosphate	11·0
Sodium chloride	2·5
Chemical analysis	
Crude protein (nitrogen × 6·25)	185
Crude fibre	56
Light petroleum extract	55
N-free extract	654
Ash	50
Calcium	8·0
Phosphorus	6·0
Sodium	1·0
Potassium	7·0
Magnesium	1·9
Zinc (mg/kg DM)	89·0
Copper (mg/kg DM)	145·0

* Parkhill No. 2 (I. Spencer Ltd, Farburn Industrial Estate, Dyce, Aberdeen) was used and provides (/kg diet): 661 μg retinol (Cu-stable), 15 μg cholecalciferol (Cu-stable), 2 mg riboflavin, 5 μg cyanocobalamin, 100 mg calcium DL-pantothenate, 10·0 mg nicotinic acid, 200 mg Cu, 100 mg Zn, 10 mg Mg, 30 mg manganese, 60 mg iron, 0·9 mg cobalt and 1·0 mg iodine (stabilized).

experiments are described in which pigs and rats were fed on a diet containing 300 g linseed meal/kg, and tests were carried out to assess the vitamin B_6 status of both species.

EXPERIMENTAL

Animals and diets

Pigs. Four castrated male Large White litter-mates, 13 weeks old and weighing 24·5–27·5 kg, were housed in metabolism cages designed for the separate collection of faeces and urine. They were given the diet shown in Table 1. The diet was considered to meet the requirements suggested by the Agricultural Research Council (1967) with the possible exception of vitamin B_6; iodine was included to counteract any goitrogenic effect of the linseed meal. The composition of the diet as found by chemical analysis is shown in Table 1.

Rats. Twelve Hooded male rats of the Rowett Research Institute strain (Rowett Research Institute, Bucksburn, Aberdeen), aged 20 d and weighing 35–40 g, were caged individually. Six were selected at random and given the 'pig' diet supplemented with 100 mg α-tocopherol and 9·4 g sodium chloride/kg diet to increase the sodium level to that suggested by Cuthbertson (1957); the remaining six rats were given the same diet with additional pyridoxine hydrochloride at the rate of 7 mg/kg diet initially, increasing to 10 mg/kg diet during the last 2 weeks of the experiment.

Experimental procedure

Pigs. The pigs were fed once daily on dry feed; after 1·5 h the food trough was removed and water was provided. This procedure was adopted to minimize possible synthesis of vitamin B_6 by fermentation and to avoid contamination of faeces and urine with food.

The pigs in metabolism cages were maintained in a temperature-controlled room at 20–25° during the 8-week experiment, the first 2 weeks of which was a period of adjustment. Two pigs received the basal diet alone and two pigs received the same diet with a supplement of pyridoxine hydrochloride (70 mg/week, given in two equal doses); the pyridoxine solution was added to a small amount of the diet which was consumed before the remainder was fed. After 5 weeks the supplement was increased to 100 mg/week. Live weight and food and water consumption were recorded throughout the experiment; faeces and urine were collected and measured for the determination of digestibility of dry matter (DM) from day 46 to day 56, protein efficiency ratio (weight gain:protein intake; PER) during the last 35 d of the experiment, nitrogen balance from day 51 to day 56 and tryptophan-metabolite excretion. Blood samples were taken at weekly intervals from the anterior vena cava.

Rats. Measurements of live weight, food consumption, food refusals and N balance were made during the last 30 d of the experiment; water was given *ad lib.*

Estimations of the PER of the pig diet were made using rats by the method of Campbell (1963), except that the protein level was 185 g/kg; dilution to 100 g/kg would have reduced any antivitamin B_6 activity of the diet. The PER was measured over the last 30 d of the 56 d experiment.

Analytical methods

Faeces and urine samples were stored at −20° before analysis. DM was estimated by drying at 100° overnight. N was determined by the Kjeldahl method.

Vitamin B_6 was estimated by the microbiological assay of Stokes, Larsen, Woodward & Foster (1943) using the mould *Neurospora sitophila* X-ray mutant 299 (I.M.I. 21944, Commonwealth Mycological Institute, Kew, Surrey) with the modifications to the culture media proposed by Barton-Wright (1945), Short & Fairbairn (1962) and Tatum, Richey, Cowdry & Wicks (1946). Vitamin B_6 was released from the dietary components by autoclaving in 0·055 M-HCl for 4 h as recommended by Freed (1966). Pyridoxine hydrochloride was used for the standard curve.

Packed cell volume (PCV) and haemoglobin in blood were estimated by the methods of Archer (1965); serum aspartate aminotransferase (*EC* 2.6.1.1) and alanine aminotransferase (*EC* 2.6.1.2) were measured by the spectrophotometric assays of Karmen, Wroblewski & La Due (1955) and Wroblewski & La Due (1956) respectively, using test kits purchased from Boehringer, Bell Lane, Lewes, Sussex BN7 1LG.

Tryptophan-load tests

The tryptophan-load test has been used to assess the vitamin B_6 status of dogs, rats and humans (Sauberlich, 1968), where the excretion of xanthurenic acid (XA)

before and after a test dose of tryptophan has provided a convenient index. Self, Brown & Price (1960) found that in pigs XA is only a minor metabolite of tryptophan and that the major ones included anthranilic acid glucuronide (AAG), *o*-amino-hippuric acid (*o*AH) and N^{α}-acetylkynurenine (AcKy).

Pigs. After the pigs had consumed the experimental diet for 45 d each animal was given 1·0 g L-tryptophan in a small amount of food; after it was consumed they were given the remaining food. Urine was collected for 24 h before and after the tryptophan test meal and stored at −20° for analysis. A second test was carried out 10 d later.

XA was estimated by the colorimetric method of Wachstein & Gudaitis (1952) as given by Sauberlich (1967). To estimate AAG, *o*AH, AcKy and kynurenine (Ky) in urine the method of Brown & Price (1956) modified by Price, Brown & Yess (1965), which was further modified was used: the tryptophan metabolites were absorbed on Dowex 50W × 12 (H-form), 200–400 mesh and eluted stepwise with hydrochloric acid at 0·1, 0·5, 1·0, 2·4 and 5·0 M to separate fractions containing indoxyl sulphate, AAG, *o*AH, AcKy and Ky respectively. The eluate containing AAG was hydrolysed in 0·05 M-HCl for 1 h at 95° and that containing *o*AH was hydrolysed in 1·0 M-HCl for 1 h at 95°; the anthranilic acid released was neutralized to pH 7·0 in phosphate buffer and measured fluorimetrically using a spectrofluorimeter (Aminco Bowman; American Instrument Co. Inc., 8030 Georgia Avenue, Silver Spring, Maryland, USA); wavelengths used for excitation and emission were 305 and 394 nm respectively.

The AcKy fraction was hydrolysed to Ky by heating in 2·4 M-HCl for 1·5 h at 95° as suggested by Brown & Price (1955); the released Ky was measured by the colorimetric method of Price *et al.* (1965). The latter procedure was also used to measure Ky in the '5·0 M-HCl' eluate. Paper chromatography of the 'Ky' eluate indicated the presence of AcKy; this was removed by increasing the volume of 2·4 M-HCl used by Brown & Price (1956) (80 ml) to 130 ml.

Rats. After 36 d on the diets, tryptophan-load tests were undertaken and the rats were transferred to all-glass individual metabolism cages. After 24 h-urine samples had been collected, 1·0 mg L-tryptophan/g body-weight was given in a small amount of diet which was followed by the remainder of the diet and a further 24 h-urine sample was collected. In order to obtain sufficient volume for analysis, the urine samples were pooled in pairs at random.

The urine was assayed for XA and kynurenic acid (KA) by the fluorimetric method of Satoh & Price (1958).

Statistical analysis

Student's *t* test was used to test the significance of the differences between mean values of non-pyridoxine-supplemented (NPS) and pyridoxine-supplemented (PS) groups of animals.

Table 2. *Nitrogen balance (g/d) and nitrogen and dry matter* (DM) *digestibility values for pigs given a diet containing* 300 *g linseed meal/kg with* (*PS*) *and without* (*NPS*) *a pyridoxine supplement**

(N balance: 5 d from day 51; digestibility trial: 10 d from day 46)

						Digestibility	
Pig no.	Treatment†	N Intake	Faecal N	Urinary N	N Retained	N	DM
1	PS	31·6	7·6	14·2	9·75	0·76	0·83
2	PS	35·6	8·9	16·1	10·62	0·75	0·83
3	NPS	26·8	4·7	18·5	3·46	0·82	0·85
4	NPS	27·1	5·4	19·7	2·04	0·80	0·86

For N retained, values for NPS pigs were statistically significantly lower than those for PS pigs ($P < 0·01$). * For details, see Table 1.

† For details see p. 323.

RESULTS

The vitamin B_6 activity of dietary components (mg/kg) was 12·0, 1·5 and 3·5 for linseed meal, oats and barley respectively: because the assay was unsatisfactory when applied to the basal diet, the value was obtained by calculation (5·4 mg/kg). Throughout the experiment the pigs were examined for signs of vitamin B_6 deficiency such as vomiting, epileptic fits or convulsions, as found by Wintrobe *et al.* (1943) and Miller, Schmidt, Hoefer & Luecke (1957); the pigs' health appeared satisfactory and on no occasion were signs of vitamin B_6 deficiency visible. Likewise the rats appeared healthy and showed no signs of dermatitis (acrodynia) or convulsions as described by Robinson (1966). Inspection of the food refusals suggested that the rats tended to reject the larger particles of linseed meal.

The apparent digestibility of DM by pigs during a 10 d collection period is shown in Table 2. Digestibility of the DM was slightly better than an estimated value obtained from published results: certainly no reduction in digestibility was apparent.

Growth rate, shown in Table 3, was slower than is normal for young growing pigs. During the last 5 weeks of the experiment the mean daily live-weight gain was 392 g and 186 g respectively for the PS and NPS groups.

The results of the 5 d N balance and the digestibility of N for pigs are shown in Table 2. The N retained by the NPS pigs was significantly less ($P < 0·01$) than that retained by the PS pigs.

PER values obtained from the rats are shown in Table 3. The difference between the two groups was not statistically significant. PER values were significantly lower ($P < 0·05$) for the NPS pigs than for the PS pigs.

Weekly estimations of PCV and blood haemoglobin in pigs are shown in Figs 1 and 2 respectively. Both sets of results show that values for the NPS pigs tended to decrease relative to those for the PS group.

Weekly measurements of aspartate aminotransferase and alanine aminotransferase activity in the serum of pigs showed values within the ranges 2–15 m units/ml and 3–17 m units/ml respectively. The results showed little consistent trend, though in the last sample taken the NPS pigs had lower values for both enzymes than the PS group.

Table 3. *Protein efficiency ratio (weight gain:protein intake;* PER*) of rats and pigs given a diet* containing* 300 *g linseed meal/kg with (PS) and without (NPS) a pyridoxine supplement*

(Mean values for six rats/group during a 30 d period and individual values for pigs during a 35 d period)

	Treatment†	Wt gain	Protein intake	PER
		(*a*) Rats		
		(g)	(g)	
	PS	114·7	61·9	1·85
	NPS	99·2	55·4	1·79
		(*b*) Pigs		
Pig no.		(kg)	(kg)	
1	PS	12·12	6·25	1·94
2	PS	15·29	7·02	2·18
3	NPS	7·53	4·46	1·69
4	NPS	5·48	3·71	1·48

For PER, values for NPS pigs were statistically significantly lower than those for PS pigs ($P < 0.05$).
* For details, see Table 1.
† For details see p. 323.

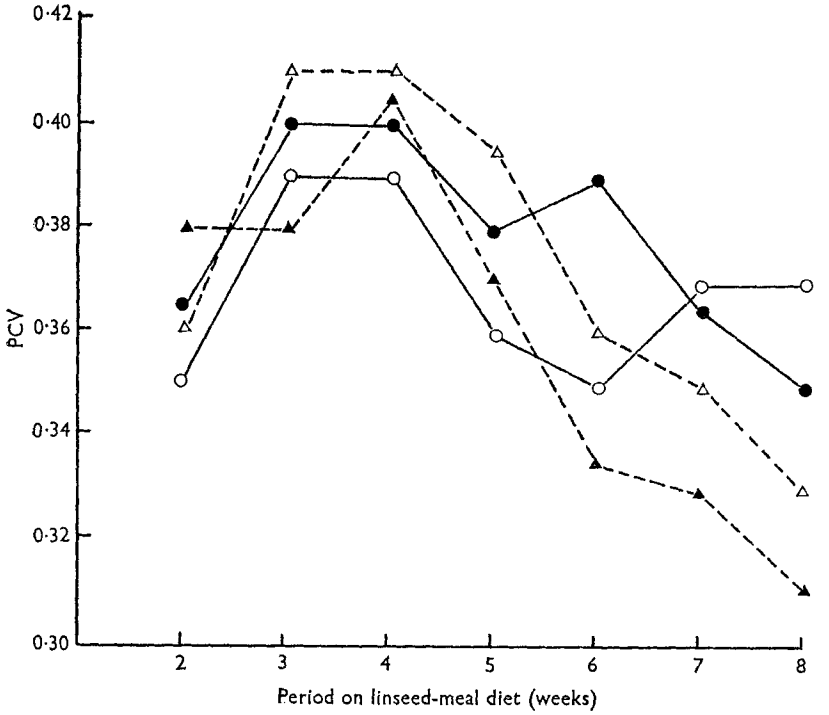

Fig. 1. The effect of a pyridoxine supplement on the packed cell volume (PCV) of pigs on a linseed-meal diet. With pyridoxine supplement: (○—○), pig no. 1; (●—●), pig no. 2; without pyridoxine supplement: (△—△), pig no. 3; (▲—▲), pig no. 4. For details of diet, see Table 1.

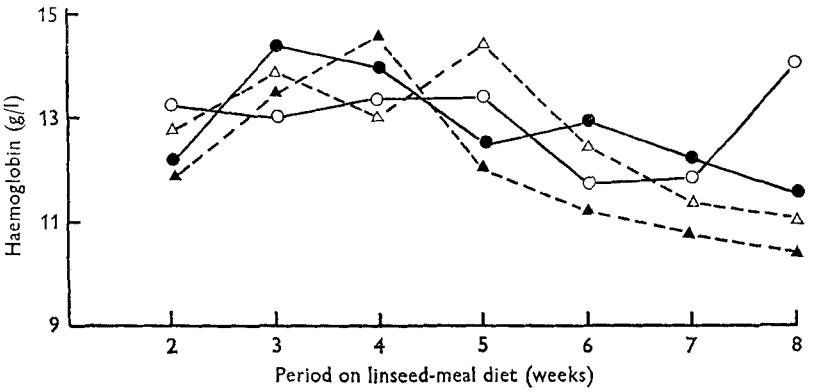

Fig. 2. The effect of a pyridoxine supplement on the haemoglobin level (g/l) in blood of pigs on a linseed-meal diet. With pyridoxine supplement: (○—○), pig no. 1; (●—●), pig no. 2; without pyridoxine supplement: (△—△), pig no. 3; (▲—▲), pig no. 4. For details of diet, see Table 1.

Table 4. *Xanthurenic and kynurenic acid levels (μmol/24 h) in urine before and after* L-*tryptophan loading (1 mg/kg body-weight) in rats given a diet* containing 300 g linseed meal/kg, with (PS) and without (NPS) a pyridoxine supplement*

(The tryptophan-load test was done after day 36 of the experiment; 24 h-urine samples of two rats were combined for analysis)

		Xanthurenic acid		Kynurenic acid	
Rats nos.	Treatment†	Before load	After load	Before load	After load
1+2	PS	2·9	17·0	0·3	2·4
3+4		5·9	13·2	0·6	1·8
5+6		3·4	18·5	0·5	2·3
7+8	NPS	1·2	15·5	0·5	4·0
9+10		2·3	13·5	1·0	2·8
11+12		1·6	18·2	1·0	2·9

* For details, see Table 1. † For details see p. 323.

The amounts of XA and KA excreted by the rats during the 24 h urinary collection, before and after tryptophan loading, are shown in Table 4. There was an increase in the excretion of both metabolites after tryptophan loading, but the difference in increased excretion between NPS and PS rats was not statistically significant.

The amounts of tryptophan metabolites in the 24 h urinary collection, before and after administering 1·0 g L-tryptophan to each pig on two occasions, are shown in Table 5. On the first occasion after tryptophan loading, the NPS group of pigs had a greater increase in excretion of XA than the PS group; this increase was highly significant ($P < 0·01$). There were no significant differences between the two treatments for the increases in AAG, *o*AH, AcKY or KY. On the second occasion the increase in XA excretion of the NPS group was again significantly greater than that for the PS group ($P < 0·02$). The NPS pigs had a greater excretion of KY than the

Table 5. *Tryptophan metabolite levels (μmol/24 h) in urine before and after* L-*tryptophan loading (1·0 g) in pigs given a diet* containing 300 g linseed meal/kg with (PS) and without (NPS) a pyridoxine supplement*

(Two tryptophan-load tests were done on days 45 and 55 of the experiment respectively; 24 h-urine samples were collected)

Pig no.	Treatment	Stage of experiment when urine collected†	Tryptophan metabolites AAG	*o*AH	AcKy	Ky	XA
1	PS	1st test: Before	10·1	6·4	141·0	51·0	65·9
		After	4·8	10·6	554·0	202·0	65·6
		2nd test: Before	54·3	84·8	137·0	43·4	102·0
		After	70·2	264·0	511·0	89·3	115·0
2	PS	1st test: Before	77·3	30·2	292·0	94·3	103·0
		After	62·2	69·5	688·0	145·0	134·0
		2nd test: Before	58·8	93·7	148·0	49·7	155·0
		After	43·7	156·0	272·0	65·6	161·0
3	NPS	1st test: Before	49·3	99·9	437·0	81·9	149·0
		After	45·9	165·0	1270·0	271·0	589·0
		2nd test: Before	37·8	58·3	189·0	47·6	191·0
		After	41·4	53·8	745·0	134·0	1540·0
4	NPS	1st test: Before	29·6	16·9	121·0	34·6	122·0
		After	41·0	90·1	681·0	153·0	472·0
		2nd test: Before	22·9	20·1	143·0	26·8	167·0
		After	31·0	69·9	560·0	142·0	1070·0

For XA, the increase after tryptophan-loading was statistically significantly greater for NPS pigs than for PS pigs: 1st test $P < 0·01$, 2nd test $P < 0·02$; for Ky, the increase after tryptophan-loading was statistically significantly greater for NPS pigs than for PS pigs: 2nd test $P < 0·05$.

AAG, anthranilic acid glucuronide; *o*AH, *o*-aminohippuric acid; AcKy, N^{α}-acetylkynurenine; Ky, kynurenine; XA, xanthurenic acid.

* For details, see Table 1.

† For details of experimental procedures, see p. 324.

PS pigs; this effect was significant ($P < 0·05$). There were increases in excretion of AAG, *o*AH and AcKy on the second occasion, which showed no significant difference between the two treatments.

DISCUSSION

Although nutritive failures occurred when a diet containing 300 g linseed meal/kg was fed by Kratzer (1946) and Kratzer, Williams, Marshall & Davis (1954) to chicks and by Tjostem *et al.* (1963) to rats, the pigs and rats used in our study did not respond in a comparable manner since neither species showed clinical signs of vitamin B_6 deficiency.

The reasons for the differences in results are not clear, but differences in the vitamin B_6 content of the ration and in the amount of antagonist present would contribute to the severity of deficiency.

The difference between the present result for rats and pigs consuming the same diet may be in part caused by two factors. The rejection of larger particles of linseed meal by the rats caused a lower intake of the vitamin B_6 antagonist. The vitamin B_6

requirement of the rat, 1–2 mg/g diet (Cuthbertson, 1957), is lower than that of the pig, 2·5 mg/g diet (Agricultural Research Council, 1967), but it is similar to the value of 1·1 mg/kg for pigs proposed by the (US) National Research Council (1964). Whether significant intestinal synthesis of vitamin B_6 in one or both species occurred is not known, but it would seem unlikely in view of the fact that the inhibitory effect on bacteria of a high dietary copper content is not accompanied by a higher dietary requirement of vitamin B_6.

A further possible reason for differences in results between groups of workers is that linseed meals may differ in their content of linatine; a range of 20–100 mg linatine/kg linseed meal has been reported by Mickelson & Yang (1966) and Klosterman *et al.* (1967).

The apparent digestibility of DM and N of the 'pig' diet showed little difference between the PS and NPS groups of pigs. As vitamin B_6 is required for amino acid absorption from the intestine, and its subsequent metabolism and incorporation into haemoglobin and proteins (see Holtz & Palm, 1964; Sauberlich, 1968), a deficiency might have influenced these processes. Moustgaard (1952) showed that vitamin B_6-deficient pigs were unable to utilize dietary protein efficiently. The greater N balance with the PS pigs than with the NPS pigs may be attributable partly to the greater food consumption and N intake by the former group, and also to decreased urinary excretion. The known role of vitamin B_6 in amino acid metabolism together with evidence of more efficient metabolism of tryptophan by the PS pigs (see Table 5) may indicate that greater economy in utilization of the absorbed N has contributed to better N retention by the PS pigs. PCV values of the NPS group of pigs tended to be lower than those for the PS pigs and ultimately decreased to less than 0·35, which Wintrobe *et al.* (1943) suggested was indicative of vitamin B_6 deficiency in pigs.

The haemoglobin values of the NPS group decreased to levels lower than those of the PS group towards the end of the experimental period, and were slightly higher than values obtained by Harmon, Miller, Hoefer, Ullrey & Luecke (1963) for vitamin B_6-deficient pigs, but were not as low as the value of 67 ± 5 g/l reported by Miller *et al.* (1957) for severely deficient pigs.

The serum aspartate aminotransferase and alanine aminotransferase activities did not show any consistent trend indicative of vitamin B_6 deficiency, even though these enzymes require pyridoxal phosphate and there is a decrease in their levels in the serum of vitamin B_6-deficient pigs (Kirchgessner & Friesecke, 1961) and rats (Brin, Tai, Ostashever & Kalinsky, 1960; Beaton & Cheney, 1964). In man the serum level of these enzymes is not a good index of vitamin B_6 status (Sauberlich, 1968).

Korbitz, Price & Brown (1963) found that after a tryptophan load, vitamin B_6-deficient rats showed an almost fivefold increase in XA excretion compared with animals receiving adequate vitamin B_6. On this criterion the rats in the present experiment appeared to be normal. Somewhat different results to those reported here were obtained by Self *et al.* (1960) who carried out tryptophan-load tests on vitamin B_6-adequate pigs which weighed 16 kg less than those used by the present authors, and were of a different breed. Moreover, tryptophan was administered using a balling gun rather than by being mixed with food as in the present study. Self *et al.*

(1960) obtained a highly significant increase ($P < 0.01$) in the excretion of AAG, and significant increases ($P < 0.05$) in XA, *o*AH, AcKY, KA and fraction A (indoxyl sulphate; Price *et al.* 1965). Self *et al.* (1960) estimated XA by the fluorimetric method of Satoh & Price (1958) and not by the colorimetric method used in our laboratory for pig urine; XA results by the former method are lower than those obtained by the latter method. The difference in animals, diet and experimental conditions may have contributed to the differences in excretion of metabolites.

Sauberlich (1968) reviewed tryptophan metabolism in vitamin B_6-deficient animals and in man, and noted that an increase in XA excretion was a regular feature, even though an enzyme required for its formation contains pyridoxal phosphate. The explanation may be that in vitamin B_6-deficient rats the decrease in activity of kynureninase (*EC* 3.7.1.3) in tissue is greater than that of kynurenine transaminase (*EC* 2.6.1.7) (Henderson, Gholson & Dalgleish, 1962; Ogasawara, Hagino & Kotake, 1962), which accepts both Ky and 3-OH-Ky as substrates (Allegri & de Antoni, 1974).

Consequently the conversions of 3-hydroxykynurenine (3-OH-Ky) to 3-hydroxyanthranilic acid and of Ky to anthranilic acid are more seriously impaired than the conversion of 3-OH-Ky to XA. However, in severe vitamin B_6 deficiency the 3-OH-Ky transaminase activity is also reduced, with a consequent decrease in XA production. Although results from rats cannot be applied to pigs with certainty, the lower levels of anthranilic acid metabolism (AAG and *o*AH) and high levels of Ky + AcKy of our NPS pigs, compared with the results of Self *et al.* (1960), may suggest an impaired kynureninase activity and vitamin B_6 status of our pigs nos. 3 and 4 (NPS group). The increase in XA shown by the NPS pigs during the second tryptophan-load test was comparable to the levels found in the urine of tryptophan-dosed pigs on inadequate levels of vitamin B_6 by Moustgaard (1952), Miller *et al.* (1957) and Harmon *et al.* (1963). The latter group of workers obtained a threefold increase in XA excretion before and after the tryptophan loading of vitamin B_6-deficient pigs.

The results of this study suggest that the diet with 300 g linseed meal/kg induced some extent of biochemical abnormality in the pigs which amounted to a marginal vitamin B_6 deficiency.

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