Vitamin B_{12} content of piglets and of milk from sows fed on rations containing animal or vegetable protein

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In recent years we have attempted to produce pigs 3–5 weeks old deficient in vitamin B_{12}. The pigs were removed from the sow at 2 days of age and reared on a vitamin B_{12}-deficient synthetic-milk diet of the composition described by Neumann, Johnson & Thiersch (1950). Unlike the American workers, we were unable to produce such deficient pigs under our conditions and considered that differences in stores of vitamin B_{12} at birth might be responsible. To investigate this possibility we did an experiment in which several gilts were fed continuously for three gestations and lactations on a ration containing only vegetable protein, as normally used in America. The vitamin B_{12} content of the baby pigs produced by these animals, and the vitamin B_{12} content of their colostrum and milk were measured and compared with those of gilts fed on a normal British-type ration containing white-fish meal as the vitamin B_{12}-rich protein supplement.

EXPERIMENTAL

Pigs. In January 1957, four Large White females of similar breeding and approximately 100 lb in weight were selected. Up till then these animals had received a growing ration containing animal protein. They were put outside on pasture in movable fold units, two animals being given meal mixture no. 15 and the other two meal mixture no. 16. The composition of these mixtures, including their content of vitamin B_{12}-active compounds as measured with *Lactobacillus leichmannii*, is given in Table 1. The two groups receiving the different meal mixtures were kept in separate fold units.

The gilts were brought indoors about 10 days before they were due to farrow and thenceforth remained indoors continuously on concrete both for that lactation period and for the following two gestations and lactations. They received the appropriate experimental meals throughout the experiment, 6 lb/day being given during pregnancy, with a further addition during lactation of $\frac{3}{4}$ lb/day for each piglet in the litter. Because of difficulties at farrowing, one of the dams (receiving control meal no. 15) had to be slaughtered immediately after she produced her second litter. Information about the second and third litters and lactations was available, therefore, for only three of the original four gilts.

Samples of colostrum were collected at each farrowing, and at 1 and 2 weeks after farrowing as much milk as possible was removed from three or four glands after

intravenous injection of 5 i.u. oxytocin. During the third lactation, samples of milk were taken also at 4 and 6 weeks after farrowing. All the samples were stored at −20° until they were assayed for vitamin B_{12} activity.

Table 1. *Percentage composition of the experimental diets given to the dams*

	Meal mixture no. 15	Meal mixture no. 16
Barley meal	52	46·75
Fine miller's offal	38	35
White-fish meal	10	—
Soya-bean meal (extracted)	—	16
Limestone	—	0·5
Steamed bone flour	—	1·5
Salt	—	0·25
Crude protein*	16·8	16·8
Vitamin B_{12} (mμg/g)	4·6	0·5

To each mixture 4·5 g/100 lb diet of Rovimix (Roche Products Ltd, containing 50000 i.u. vitamin A and 12500 i.u. vitamin D_3/g) were added.

* From mean values for the ingredients given by Woodman (1957).

Table 2. *Numbers of piglets killed and of colostrum and milk samples collected during the three successive lactations of the four dams*

Dam no.	Protein supplement in diet	Lactation	No. of piglets killed: At 2 days	No. of piglets killed: At 14 days	Sample taken*: Colostrum	Sample taken*: Milk: 1 week	Milk: 2 weeks	Milk: 4 weeks	Milk: 6 weeks
7301	Fish meal	First	2	2	+	+	+	.	.
		Second	2	2	+	+	+	.	.
		Third	1	1	+	+	+	+	+
7279†	Fish meal	First	2	2	+	+	+	.	.
		Second	.	.	+	.	.	.	.
7302	Soya-bean meal	First	2	1	+	+	+	.	.
		Second	1	2	+	+	+	.	.
		Third	1	1	+	+	+	+	+
7329	Soya-bean meal	First	2	2	+	+	+	.	.
		Second	1	2	+	+	+	.	.
		Third	1	1	+	+	+	+	+

* Shown by +.

† Slaughtered immediately after producing second litter (see p. 43).

From each litter one or two piglets were killed in an ether chamber at 2 days of age and one or two at 14 days of age. In the first two litters from each dam only those piglets due to be killed at 14 days of age were left to suckle their dams after 2 days. Those not killed at 2 days were taken away and reared artificially; the findings with these piglets will be reported in another communication. In the third litter from each dam, one piglet was killed at 2 days and one at 14 days, the remainder being left with their dams until 56 days of age. No creep feed was provided for any of the piglets left with their dams in any of the lactations.

Information about the young piglets and the colostrum and milk samples examined is given in Table 2.

Assay of vitamin B_{12}. With the first litter from each gilt, the bodies of the piglets killed at 2 and at 14 days of age were washed and the livers and alimentary canals removed. The remainder of each piglet was then minced, and a 500 g portion of the mince homogenized in a Waring Blendor with an equal weight of water. Similarly, the liver and alimentary canal (complete with contents) were separately homogenized with their own weight of water. Samples of these homogenates (10 g) were diluted with about 75 ml water containing 0·4 ml 1% (w/v) NaCN solution, the pH was brought to 5·0 with N-HCl and the sample heated in flowing steam for 30 min. After cooling, the samples were made with water to a volume of 100 ml and filtered. The clear filtrates were diluted further to contain about 0·04 mμg vitamin B_{12}/ml and their vitamin B_{12} content was measured with *L. leichmannii* A.T.C.C. 4797 as described by Gregory (1954). With the second and third litters from each dam, only the vitamin B_{12} content of the livers of the piglets killed at 2 and at 14 days of age was measured.

All the samples of colostrum and sow's milk were prepared for measurement of vitamin B_{12} activity by the papain-digestion method described by Gregory (1954).

The vitamin B_{12} content of the diets was measured as follows: 5 g samples of meal mixtures nos. 15 and 16 were suspended in 30 ml water, 10 ml 0·1 M-sodium-acetate buffer at pH 4·6 and a drop of 1% (w/v) NaCN solution were added and the whole was heated at 100° for 30 min. After cooling, the extracts were made up to 100 ml with water and filtered. Portions of the clear filtrates were: (*a*) diluted without further treatment to contain about 0·04 mμg vitamin B_{12}/ml, and (*b*) treated with alkali as described by Gregory (1954). The vitamin B_{12} activity of the two solutions was measured with *L. leichmannii*. As alkali treatment inactivates vitamin B_{12}, any growth activity remaining after such treatment is due to substances, other than vitamin B_{12} or its analogues, that are active for *L. leichmannii* (e.g. deoxyribosides).

RESULTS AND DISCUSSION

Results of the microbiological assays of the vitamin B_{12} content of the samples of colostrum and milk are given in Table 3, and those for the liver, intestine (including contents) and body (without liver and intestine) of the piglets from the first litters of the four gilts are given in Table 4. In Table 5, the vitamin B_{12} content of the liver of piglets from the second and third litters of the dams is recorded. The values given in Tables 4 and 5 for the vitamin B_{12} content of livers of 2-day-old piglets are somewhat lower than values previously found for newborn piglets from sows in our herd kept continuously on pasture and fed on a diet containing animal protein (unpublished results). They are also lower than those reported by Bauriedel, Hoerlein, Picken & Underkofler (1954); no details of the management and diet of the dams of the piglets are, however, given by these workers.

The values in Tables 3–5 indicate no consistent effect of the diet of the dams on the content of vitamin B_{12} either in their colostrum or milk, or in the bodies of their offspring. Although diet no. 16, containing soya-bean meal as the protein supplement,

had only about one-tenth of the vitamin B_{12} activity of diet no. 15 (see Table 1), in the amounts given it probably supplied about 1·4 μg vitamin B_{12}/day during each pregnancy. The requirement of pregnant adult pigs for vitamin B_{12} has not yet been established, but this estimated daily intake may be compared with the value of about 1 μg vitamin B_{12} considered as the approximate daily requirement of adult man.

It is evident that the largest drain of vitamin B_{12} is normally in the milk secreted during the standard 8-week lactation period. Some indication of the probable extent of this loss during lactation of the two animals that received the soya-bean diet is

Table 3. *Vitamin B_{12} activity (mμg/ml) of the colostrum and milk of the four dams*

Dam no.	Protein supplement in diet	Lactation	Colostrum	Milk 1 week	Milk 2 weeks	Milk 4 weeks	Milk 6 weeks
7301	Fish meal	First	0·8	1·8	1·4	—	—
		Second	0·5	0·8	2·2	—	—
		Third	0·9	1·4	1·5	1·2	1·4
7279*	Fish meal	First	2·4	2·0	2·6	—	—
		Second	0·6	—	—	—	—
7302	Soya-bean meal	First	1·3	1·7	2·0	—	—
		Second	0·8	0·9	1·2	—	—
		Third	0·9	1·7	1·5	1·1	1·1
7329	Soya-bean meal	First	3·2	2·7	3·3	—	—
		Second	0·9	1·5	1·6	—	—
		Third	1·4	1·8	1·8	2·0	1·7

* Slaughtered immediately after producing second litter (see p. 43).

Table 4. *Vitamin B_{12} activity of the liver, intestine (including contents) and body (without liver and intestine) of piglets from the first litters of the four dams*

Dam no.	Protein supplement in diet	Piglet No.	Piglet Age (days)	Vitamin B_{12} activity: Liver mμg/g	Liver Total (μg)	Intestine mμg/g	Intestine Total (μg)	Body mμg/g	Body Total (μg)	Body and liver Total (μg)
7301	Fish meal	1	2	27·0	1·8	11·0	1·6	1·6	2·2	4·0
		2	2	40·0	2·0	8·5	0·7	2·5	2·0	4·0
		3	14	21·0	3·9	27·0	9·4	1·9	6·8	10·7
		4	14	20·0	2·7	44·0	12·0	1·9	5·6	8·3
7279	Fish meal	1	2	50·0	2·9	10·0	1·4	3·0	3·0	5·9
		2	2	50·0	2·1	20·0	2·3	3·0	2·8	4·9
		3	14	30·0	•	40·0	•	3·0	•	•
		4	14	30·0	•	40·0	•	2·0	•	•
7302	Soya-bean meal	1	2	60·0	1·5	14·0	1·0	3·6	2·5	4·0
		2	2	42·0	1·8	8·0	1·0	2·2	2·4	4·2
		3	14	14·0	2·6	11·0	3·7	1·5	4·2	6·8
7329	Soya-bean meal	1	2	80·0	3·6	10·0	1·2	5·0	4·7	8·3
		2	2	80·0	3·6	10·0	1·0	6·0	4·8	8·4
		3	14	40·0	8·4	30·0	12·3	3·0	10·5	18·9
		4	14	60·0	9·1	30·0	9·8	4·0	12·6	21·7

• Values for weights of body, liver and intestines were lost.

given by calculation of the approximate output of vitamin B_{12} in the milk of dams nos. 7302 and 7329 from the figures in Table 3 for vitamin B_{12} activity and the values of Barber, Braude & Mitchell (1955) for estimated milk yields. For dam no. 7302, the values obtained in this way for the first, second and third lactations were around 60, 30 and 400 μg vitamin B_{12}, respectively, and for dam no. 7329, the corresponding values were 130, 40 and 800 μg. The markedly higher total output of vitamin B_{12} in the milk in the third lactation of each dam is striking and arises from the fact that in

Table 5. *Vitamin B_{12} activity of the liver of piglets from the second and third litters of three of the dams*

Dam no.	Protein supplement in diet	Lactation	Piglet No.	Piglet Age (days)	Vitamin B_{12} activity mμg/g	Vitamin B_{12} activity Total (μg)
7301	Fish meal	Second	1	2	18·0	0·8
			2	2	18·0	1·5
			3	14	8·8	1·8
			4	14	7·9	2·0
		Third	1	2	32·0	1·5
			2	14	39·5	4·2
7302	Soya-bean meal	Second	1	2	10·0	0·7
			2	14	9·4	1·4
			3	14	8·2	1·2
		Third	1	2	32·5	2·1
			2	14	26·5	3·7
7329	Soya-bean meal	Second	1	2	25·0	2·3
			2	14	18·0	4·4
			3	14	16·0	3·4
		Third	1	2	35·5	2·6
			2	14	22·0	3·6

that lactation only two piglets were killed from each litter, all the remaining piglets being allowed to suckle their dams for 8 weeks, whereas in the first two lactations all the piglets were removed from the dam by 2 weeks of age.

Consideration of these figures in relation to the probable intake of vitamin B_{12}, as supplied by meal no. 16, indicates that if the majority of the piglets born in the first and second litters had been allowed to suckle their dams for the normal 8 weeks some reduction in vitamin B_{12} activity in the milk and in the newly born piglets might have been observed during the experimental period. This is, however, mere speculation, since there are no data available for an estimate of any vitamin B_{12} obtained from the soil by the gilts while on pasture, or of the extent of microbial synthesis of vitamin B_{12} in the intestine and its subsequent absorption and utilization by the dams.

In connexion with this question of the drain of vitamin B_{12} reserves through the milk secreted, it should be remembered, however, that under American conditions breeding stock are usually retained for one or possibly two lactations only. They are also normally kept on soil, and breeds other than Large White are used. Taking account of these differences and of the further possibility that breeds may differ in the economy

of utilization of vitamin B_{12}, we cannot at present say to what extent the production of vitamin B_{12} deficiency in piglets is influenced by their body stores of the vitamin at birth.

SUMMARY

1. The effect of the diet of the dam on the vitamin B_{12} stores of newborn piglets has been investigated.

2. Two Large White gilts received a diet with white-fish meal and two received an all-vegetable diet.

3. The vitamin B_{12} contents of 2-day- and 14-day-old piglets and of colostrum and milk were measured during three gestations and lactations.

4. No effect of the diet of the dams on the content of vitamin B_{12} in their piglets, colostrum or milk was found.

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