THE EFFECT OF DIETARY BIOTIN LEVEL ON THE PRODUCTIVITY OF THE FEMALE PIG

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A B S T R A C T

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Five experiments were conducted with female pigs to investigate the effects of dietary biotin level on: reproductive performance and hoof integrity over four parities (experiment 1); ovulation rate of the gilt (experiment 2); durability of hoof horn and the phospholipid and neutral lipid profile of perinephric and hoof horn fat (experiments 3 and 4) and milk fat (experiment 5). Experiment 1 showed that changes in reproductive performance and hoof integrity in adult sows occurred when pigs were fed levels of dietary biotin previously considered to have been sufficient to meet the sow's requirements (diet calculated to provide 32μg available biotin/kg). Notably, sows receiving 350μg supplementary biotin/kg returned to oestrus 2.9 ± 1.7 and conceived 6.1 ± 1.4 days sooner than controls ($p < 0.05$). The number of lesions/sow increased greatly between 170 days of age and first weaning, at which time the control sows had significantly more lesions/sow (13.45 v 9.79; $p < 0.001$), but appeared to stabilise in the oldest sows. The production of unsaturated fatty acids in the neutral lipid fraction of the milk increased between early and late lactation in the supplemented but not control sows ($p < 0.05$) in a sample of sows from control and supplemented treatments respectively (experiment 5). The effects on reproductive performance and the biochemical and physical effects observed in the growing pig indicated that biotin deficiency may produce commercially significant effects prior to the development of symptoms of clinical deficiency. No treatment effects were observed for weight of ovary or number of corpora lutea produced by gilts (experiment 2). Hoof horn durability, measured using a Durometer, was greatest in gilts fed high levels of dietary biotin (experiment 4). The fractionated analyses of the perinephric fat indicated that the relative percentage of C16:0 and C18:0 compared to C16:1 and C18:1 increased with greater dietary biotin intake and analyses of hoof horn fat indicated similar trends (experiments 3 and 4).

CONTENTS

FIGURES

LITERATURE REVIEW

INTRODUCTION

The farming industry has undergone profound changes this century in
meeting the increasing demands of an expanding human population. It
has seen the introduction of a more industrially organised production
into livestock husbandry. The resulting intensification has demanded
a greater understanding of the genetic selection of stock, housing con-
ditions, hygiene and regulated feeding systems. Previously, nutrition-
al demands of stock were not always satisfied by seasonally-produced
feedstuffs upon which they were dependent. In overcoming these
seasonal constraints, modern feeding systems have restricted free
choice of feed in favour of manufactured feeds, with the aim of lower-
ing feed consumption per unit of production. Here genetic selection of
the stock and increased nutrient density of the feed have played a part,
requiring detailed knowledge both of an animal's nutritional require-
ment and of the composition and nutritive value of the feedstuffs to
ensure maximum productivity.

All stock require a regular intake of some forty different dietary com-
ponents which are "essential" to the health of the animal. They com-
prise not only the energy-yielding and tissue-building substances
(proteins, fats, carbohydrates, amino-acids and mineral salts) but also
micronutrients (vitamins and trace elements). Vitamins are required
daily in very small amounts (microgrammes or milligrammes). The
vitamins act as cofactors in enzyme systems, thereby controlling
metabolism by synthetic and degradative processes without serving as
building substances themselves. Should a vitamin be present in
inadequate amounts, the impairment of the animal's metabolic process
would lead to disturbances in productivity, growth inhibition and
disease. Sometimes a single biochemical reaction is affected resulting
in characteristic symptoms, but often several metabolic reactions are

affected giving a confused pattern of disturbed health.

In addition, there is a graded response to the level of deficiency, from clearly defined symptoms (avitaminosis), to less defined symptoms such as lower reproductive performance (hypovitaminosis) and deficiency symptoms occurring following sudden stress (latent hypovitaminosis). However the vitamin requirements of stock are continually varying in response to their changing environment. The diet may contain anti-vitamins or vitamin antagonists which may inactivate a vitamin. For example, the substance avidin, which occurs in raw and dried egg white, forms a complex with biotin in the gastro-intestinal tract thereby preventing resorption of this vitamin (Baugh et al., 1968; Sydenstricker et al., 1942). Other nutrient components may alter the requirements for a vitamin involved in its metabolism by either sparing the vitamin or increasing the requirement for it. This is illustrated during the period of lactation when higher levels of biotin are necessary to support fat synthesis and excretion than during other periods of the sow's reproductive cycle. Vitamin requirements are also increased by bacteriostats (eg sulphonamides and coccidiostats) which may alter the nature of the intestinal flora which might otherwise supply a source of vitamins for the host (Ham and Scott, 1953; Welch and Wright, 1943). The activation of defence mechanisms increases metabolic activity, as does physical exertion and increased production, and so raises demand. Infected organisms and parasites compete with the host for vitamins and intestinal parasites attack the mucous membranes and interfere with vitamin resorption. Greater enzyme activity is needed to degrade and excrete the toxins produced by disease organisms, again resulting in an increased requirement for vitamins.

The feed material can also provide a source of variability of the vitamin for the stock. The vitamin contents of plants and animals can vary widely. Firstly, the factors which influence cereal vitamin content within a species are variety, geographic area of growth, season and yield (Robinson et al., 1949 and 1950; Tunger and Thomas, 1967; Herting and Drury, 1969, Scheiner and De Ritter, 1975; Putnam, 1978). Secondly, the biological availability of a vitamin differs between feedstuffs. This occurs in the case of biotin which is wholly available from maize, and is unavailable in wheat (Frigg, 1976), the enzymes of the gastric

2

tract being incapable of liberating completely the biotin bound to the cell walls in wheat.

Further difficulties in assessing the vitamin content of feedstuffs result from differences in the method of processing the feedstuff, the duration and method of storage, and in the method of extraction and assay used, which may be subject to systematic error.

Therefore to ensure a regular supply of vitamins, the addition of vitamins to the diet in a pure form has an established place in modern animal nutrition. However, it was considered that biotin from the intestinal tract, plus biotin present in a "normal" diet was sufficient to fulfil the sow's requirements and that spontaneous biotin deficiency was unlikely to occur in sows. ARC (1967) concluded there was normally "no dietary requirement" for biotin if the diets fed contained no sulpha drugs. This view was challenged as a result of phenomena observed in the Seale-Hayne College pig herd.

A group of sows from this herd spontaneously developed lameness, skin lesions and alopecia which could have been produced by several inter-relating factors (Brooks et al., 1977). The condition of the skin could have resulted from parasitic infection but investigations produced negative results. Previous work on foot lesions (Penny et al., 1965) suggested that lameness resulted when sows were moved from free-range systems to confinement on concrete floors. No such change had occurred in the case of the College herd. Foot bathing with formalin solution did not prevent the development of further cases of lameness. It was then realised that the symptoms closely resembled those reported from trials in which biotin-deficient diets had been fed (Cunha et al., 1946; Glättli, 1975), but it could not be claimed with certainty that the symptoms initially observed in the sows resulted entirely from the dietary deficiency of biotin.

Examining the hypothesis that these sows were suffering from a spontaneous deficiency of biotin, subsequent trials demonstrated that supplementation of the diets used with biotin was successful in arresting the development of some categories of foot lesions and reducing the incidence of others. An unexpected response to the increase in dietary biotin intake was the apparent improvement in the reproductive

3

performance (Brooks et al., 1977). Second parity biotin-supplemented sows produced significantly more live pigs than control sows (1.64 ± 0.77, p ⊲ 0.05). The weaning to remating interval was significantly reduced from 15.31 ± 2.55 days in the control sows to 6.23 ± 2.85 days in the supplemented sows (p ⊲ 0.05) and the percentage exhibiting oestrus within seven days was increased from 56% to 89% in the supplemented sows.

These results have to be treated with caution due to the experimental design. Firstly, sows started treatment at different stages of the reproductive cycle and secondly, whilst there were differences between the biotin-supplemented and unsupplemented groups, skin and hair condition did improve in both treatments during the course of the trial. The unsupplemented group also showed an improvement in hoof horn hardness and disappearance of lesions from some claws, but to a lesser degree than exhibited by the biotin-supplemented sows. The mean number of claws affected by lesions was reduced by 1.37 for the control sows and 2.85 for the supplemented sows. Furthermore, as the trial progressed the differential effects on reproductive performance became less marked. However, the improvement in reproductive performance, both in weaning to remating interval and in piglet production, was of particular interest as it was the first evidence from a controlled experiment to confirm earlier observations on commercial pig units by Cunha (1971).

The conclusions that may be drawn from the study of Brooks et al. (1977) are limited as the stock was not initially healthy. The relationship between the development of lameness, the effect on reproductive performance and the level of dietary biotin intake in the sow needs to be investigated further. Clearly it is a complex subject which may be better understood by a review of the following areas:

a. an outline of the biochemical role of biotin;

b. the factors affecting constraint of the availability of biotin in commercial sow rations;

c. the physiological, pathological and biochemical effects of induced biotin deficiency;

d. the responses of sows to biotin supplementation of non-synthetic

4

diets; and

e.   the techniques used for assessing the biotin status of a pig.

ROLE OF BIOTIN IN BIOCHEMISTRY

The role of biotin as an essential component of a number of specific
enzyme systems and other biochemical processes has been reviewed by
Mistry and Dakshinamurti (1964), Lynen (1967), Moss and Lane (1971),
Achuta Murthy and Mistry (1977). Biotin has both direct and indirect
effects.

The well-established role of biotin is in its participation as the
prosthetic group of enzymes that carry out carboxylation reactions.
Carboxylases catalyse energy-dependent fixation of carbon dioxide to
various substrates. Biotin, acting as a co-factor for enzyme proteins,
is capable of taking up carbon dioxide with the formation of a carbon
dioxide-biotin enzyme complex ("active carbon dioxide") and transfer-
ring it to a suitable substrate, regenerating the free biotin-enzyme
complex. Such carboxylation reactions are involved in the degradations
of amino-acids (leucine and iso-leucine) and the reversible carboxy-
lation of pyruvate from oxaloacetate which is a connecting link in the
citric acid pathway. Therefore biotin plays an essential role in the
conversion of a variety of three-carbon precursors to glucose. The
carboxylation of acetyl coenzyme A (CoA) to malonyl CoA is the initial
step in the synthesis of fatty acids and determines the rate at which
the long-chain fatty acids are formed within the body. A further car-
boxylation reaction involves the metabolism of propionate which can be
utilised for both energy derivation and glucose production. The well-
known carboxylations and their reactions are listed in Table 1.

Biotin deficiency results in the impairment of very many other reactions
in the intact organism. These reactions are not inhibited by avidin.
Since avidin specifically binds biotin, these reactions are not directly
mediated by biotin enzymes and the effects observed are indirect. It is
probable that the effective operation of the pathways is limited owing
to the reduced availability of substrates, particularly four-carbon pre-
cursors, whose synthesis depends on biotin enzymes (Achuta Murthy and
Mistry, 1977). Mistry and Dakshinamurti (1964) provided the following

5

Table 1[/]

Biotin-dependent carboxylases

Enzyme	Reaction catalysed (substrate → product)	Biochemical role
Acetyl - CoA carboxylase	Acetyl - CoA → malonyl - CoA	Fatty acid synthesis
Pyruvate carboxylase	Pyruvate → oxaloacetate	Gluconeogenesis, generation of 4-carbon intermediates (anaplerotic reactions) and lipogenesis
Propionyl - CoA carboxylase	Propionyl - CoA → methylmalonyl - CoA	Propionate metabolism in animals and microorganisms
β - Methylcrotonyl - CoA carboxylase	β - Methylcrotonyl - CoA → β - methylglutaconyl - CoA	Catabolism of leucine
Geranyl - CoA carboxylase	Geranyl - CoA → carboxylated geranyl - CoA	Bacterial degradation of isoprenoid compounds
ATP : urea amidolyase	Urea → N - carboxyurea ↓ H_2O NH_3 + CO_2	Bacterial catabolism of urea
Transcarboxylase	Methylmalonyl - CoA + pyruvate → oxaloacetate + propionyl - CoA	Bacterial propionate metabolism

[/]Achuta Murthy and Mistry (1972)

list of biochemical reactions to indicate the indirect influence of biotin on metabolism:

1. Deamination of aspartate, serine and threonine in bacteria;

2. Deamination of serine in animals;

3. Reductive carboxylation of pyruvate by the malic enzyme;

4. Carboxylation of phosphoenolpyruvate by phosphoenolpyruvate carboxylase;

5. Carbamylation reactions;

6. Tryptophan metabolism;

7. Purine synthesis;

8. Protein synthesis;

9. Carbohydrate metabolism.

More recently Boeckx and Dakshinamurti (1974) reviewed biotin-mediated protein biosynthesis and observed that biotin administration seemed to have a general stimulatory effect on both cell-free amino-acid incorporation into protein and orotic acid incorporation into nuclear RNA in the biotin-deficient rat in vivo. They concluded that the synthesis of some proteins was unaffected by biotin deficiency whilst others were reduced so that some enzyme systems not directed involving biotin had reduced activity.

It is not yet possible to account for the usual clinical signs of biotin deficiency in terms of a loss of activity of a biotin-dependent enzyme. Reproduction could be affected by such loss, but it is more likely that the varied influences of biotin on metabolism could depress the complex metabolic processes of reproduction.

AVAILABILITY OF BIOTIN IN COMMERCIAL SOW RATIONS

Modern feeding techniques allied to the constraints of the biological availability of biotin in natural feed ingredients are now thought to be the likely explanation for the occurrence of marginal biotin

7

deficiencies such as those reported by Brooks et al (1977). Brooks (1978) demonstrated that while the average performance of breeding sows had increased between 1957 and 1977, their feed intake had diminished over the same period (Figure 1). Therefore not only was less feed consumed per weaner pig produced but also the biotin provision per kilogram produced had been reduced. Concomitant with this was the growing awareness of the limited biological availability of biotin in many commercial feedstuffs.

Of the eight different stereoisomers of biotin only the dextrorotatory (d-biotin) exists in nature and has vitamin activity. It occurs partly in the free state (for example, in vegetables, fruit and rice-bran) and partly in a form of bound protein (for example, in animal tissues, plant seeds and yeast). Data reported by Lampen et al (1942) indicated that feedstuffs of plant origin contained a higher ratio of free biotin (biotin available to a test organism after water extraction) than feedstuffs of animal origin. More recently Scheiner and De Ritter (1975) also determined the relative amounts of free biotin in a number of feedstuffs of different origin (Table 2). They confirmed that feedstuffs of plant origin contained a higher percentage of free biotin than those of animal or yeast origin with the exception of poultry by-product meal.

However the relationship between the free and bound forms of biotin in feedstuffs does not necessarily indicate the biological availability of biotin to the organism. Research on available biotin content of feeds has not yielded conclusive results. Wagstaff et al.(1961) reported that wheat and barley contained very low amounts of available biotin for the chick in contrast to the high levels indicated by microbiological assays. Chick assay indicated that barley contained 35 µg biotin/kg whilst 100 µg/kg were found by microbiological assay. Scheiner and De Ritter (1975) first investigated the validity of the technique for total biotin determination by microbiological assay. The test organism was Lactobacillus plantarum (arabinosus 17-5, ATCC No. 8014) which was considered to yield the most reliable results. However as microorganisms can only utilise free biotin from feed materials, a hydrolytic procedure is necessary to liberate the bound biotin. The hydrolytic procedure was investigated by Scheiner and De Ritter (1975) who discovered that biotin

8

FIGURE 1

Change in sow productivity and feed per weaner at 8 weeks of age (1957-1977)
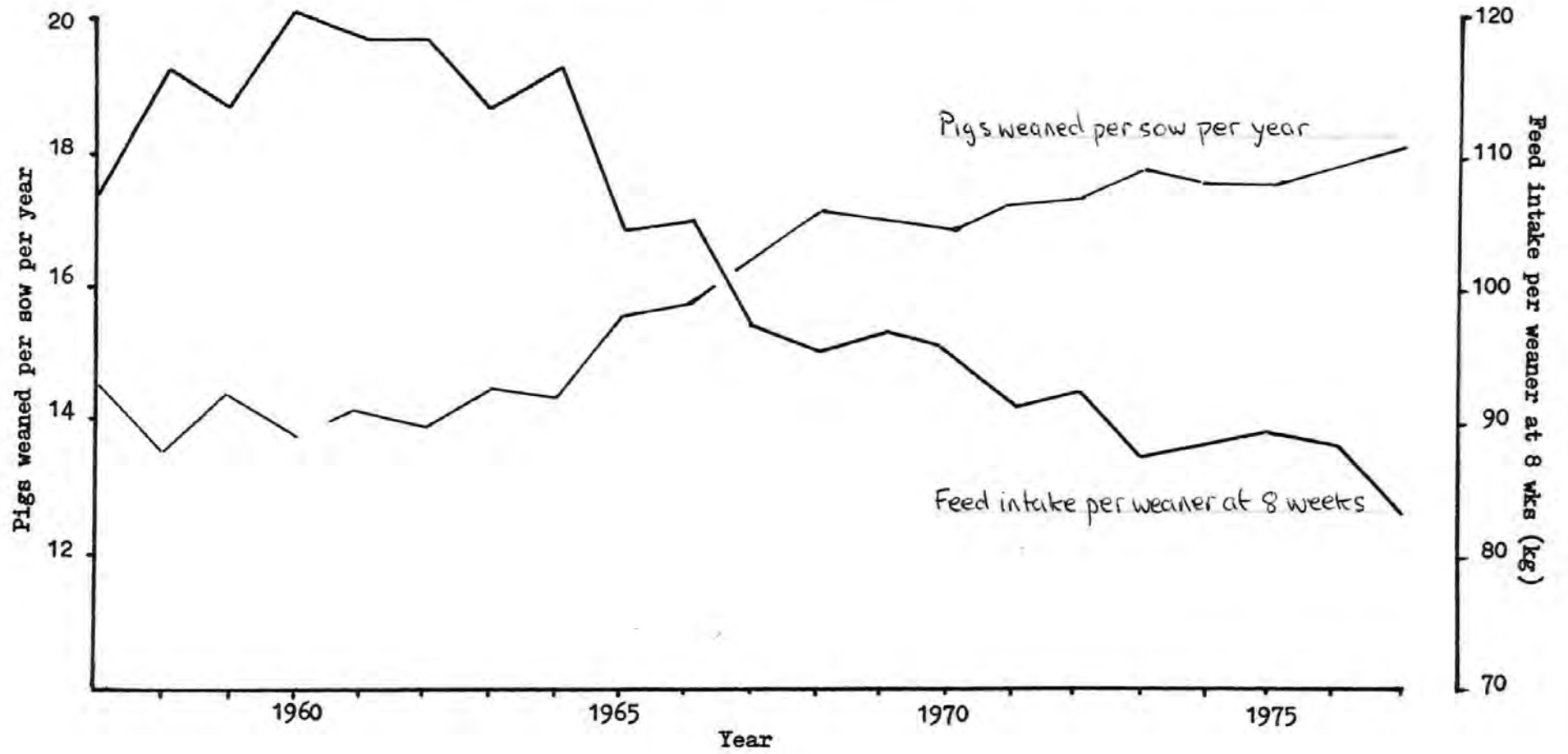
TABLE 2[/]

Free and total biotin in feedstuffs of plant and animal origin

Ingredient	Free biotin (µg/kg)	Total biotin (µg/kg)	(Free biotin/ total biotin) x 100, %
Alfalfa	520	650	80
Safflower	960	1560	62
Sorghum	75	230	33
Maize	18	80	23
Casein	14	50	28
Wheat, soft	35	98	36
Soyabean meal	100	44	23
Brewers' dried yeast	200	1360	15
Herring meal	50	45	11
Meat and bone meal	30	20	15
Poultry by-product meal	14	48	29

[/] Scheiner and De Ritter (1975)

was less stable in relation to autoclaving time and acid concentration in extraction of plant matter than in animal tissue. More efficient liberation of biotin was obtainable from feedstuffs of plant origin with 2N sulphuric acid and from feedstuffs of animal origin with 6N sulphuric acid. This technique results in higher values than may be otherwise achieved. Anderson and Warnick (1970) compared the results from microbiological assays undertaken by this method with results from chick bioassays (Table 3). Generally both the assay techniques showed the same feedstuff to be either a rich or poor source of biotin. The microbiological assays tended to give higher values than the chick estimates, although exceptions were noted. Differences were partly explained by incomplete bioavailability to the chick. They observed that the biotin in some cereal grains and in fish meal was only 25-55% available while in soya bean the biotin was 100% available. To determine the growth response of chicks to the various feedstuffs, each feedstuff was substituted for part of a purified ration which was based on casein and gelatin.

Frigg (1976) obtained a growth response curve by chicks to the biotin contained in a cereal feedstuff by substituting ground rice in the basal diet by the feedstuff under test. The microbiological assay of the biotin content was determined by the liberation of biotin in a feedstuff by hydrolysis with 2N sulphuric acid and using the same test organism as described previously. The availability of biotin in a raw material varied from over 100% in maize, 22% and lower in barley, to being not measurable in wheat (Table 4). A value in excess of 100% in maize was possible as L.arabinosus also gives a growth response to derivatives of biotin (Adrian, 1959). It is worth noting that growth of L.arabinosus can also be inhibited by biotin analogues such as biotin sulphane (Du Vigneaud, 1942). The estimates of biotin availability for some feedstuffs differed greatly from those obtained by Anderson and Warnick (1970) and Frigg (1976). For example, an estimate of 72% availability for wheat was obtained by Anderson and Warnick (1970, Table 4). The difference in estimates was explained by the fact that in the trial of Anderson and Warnick (1970) the addition of some feedstuffs to the basal diet increased the severity of symptoms of biotin deficiency although average weight gain increased. Consequently improvement in performance

11

TABLE 3[/]

Biotin content of feedstuffs

Ingredient	Chick bioassay estimate (µg/kg)	Micro-biological estimate (µg/kg)
Oilseed meals		
Soyabean meal, 50% protein	600	500
48% protein	800	400
44% protein	800	400
Cottonseed meal, 52% protein	1100	
42% protein	600	450
Peanut meal, 58% protein	1800	1800
Safflower meal, 42% protein (1)[1]	1700	1700
(2)	1700	2000
Sesame meal, 48% protein[1]	800	
Animal protein supplements		
Meat and bone meal[2]	283	233
Herring meal, 71% protein	150	400
Tuna meal, 53% protein	50	200
Hake meal, 72% protein	200	500
Peruvian fish meal, 71% protein	100	400
Poultry by-product meal, 59% protein	200	450
Grain and Grain products		
Maize gluten meal, 42% protein	250	400
Maize	125	125
Sorghum	100	250
Barley	60	125
Wheat, soft white	60	125
Wheat, hard red	90	125
Miscellaneous feedstuffs		
Dehydrated alfalfa	800	550
Alfalfa meal, lab cured	1000	
Dried yeast grown on whey	2000	1800
Brewer's dried yeast	1000	1000
Corn distillers dried solubles	400	450
Distillers dried grains with solubles[3]	400	350
Cond. fermented corn extractives	600	500

Note:

[1] Samples shelved at room temperature for over 5 years and had a rancid odour.

[2] Mean of three samples; chick bioassay for one sample was unexpectedly high and was not confirmed by the microbiological estimate.

[3] Mean of two estimates.

[/] Anderson and Warnick (1970).

TABLE 4

Biotin content of various grains by microbiological, chick bioassay and enzyme techniques

Grain	a	b	c	d	%age avail-ability[1]	e	f	%age avail-ability[2]	g	%age avail-ability[3]	h	%age avail-ability[4]	i	j	%age avail-ability[5]
Barley	110	200	125	60	48	144	29	20	82	75	95	5	109	12	11
Maize	94	80	125	125	100	45	48	107	108	87	97	103	50	65	133
Wheat	105	100	125	90	72	104	-	-	43	41	51	49	84	4	5

[a] Scheiner and De Ritter (1975) - microbiological assay.
[b] Allen (1976) - microbiological assay.
[c] Anderson and Warnick (1970) - microbiological assay.
[d] Anderson and Warnick (1970) - chick bioassay.
[e] Frigg (1966) - microbiological assay.
[f] Frigg (1966) - chick bioassay;
The chick bioassay technique was insufficiently sensitive to provide any values for wheat and gave only two values for barley from five samples.
[g] Anderson et al.(1978) - chick bioassay.
[h] Anderson et al.(1978) - hepatic pyruvate carboxylase assay;
Many of the pyruvate carboxylase values were below the intercept of the standard biotin response curve, so estimations of the biotin concentration in the grains from pyruvate carboxylase activity data were based on few observations.
[i] Whitehead et al.(1982) - microbiological assay.
[j] Whitehead et al.(1982) - blood pyruvate carboxylase assay.

[1] %age availability was calculated by dividing the result for biotin content from chick or enzyme assay by microbiological assay for the same sample where appropriate and multiplying by 100. Therefore %age availability = d/c x 100.
[2] f/c x 100.
[3] g/a x 100.
[4] h/a x 100.
No microbiological assay was undertaken by Anderson et al.(1978), therefore the microbiological assay results achieved by Scheiner and De Ritter (1975) were used to obtain an estimate of percentage availability for g and h.
[5] j/i x 100.

following addition of a feedstuff may not have been entirely due to the biotin ingested. However Whitehead et al. (1982) considered that the bioassay technique based on chick growth response had several disadvantages. Firstly, the biotin content of some feed ingredients was so low that very high inclusion levels of the feedstuffs were required to elicit a growth response. Moreover, the growth response was non-specific and may have been influenced by changes in other aspects of the diet, such as nutrient concentration or palatability.

Whitehead et al. (1982) investigated an alternative means of assessing bioavailability of biotin, based on the activity of pyruvate carboxylase (pyruvate-carbon dioxide ligase (ADP-forming) EC 6.4.1.1:PC) in the blood of young chicks. This enzyme has been shown to be closely related to the biotin status of birds and the biotin content of their diet (Whitehead and Bannister, 1978, 1980). The estimates they obtained for bioavailability of wheat and barley were low and were similar to those achieved by Frigg (1976, Table 4). The bioavailable biotin content of maize was higher than that obtained microbiologically, and at the extreme of the confidence limits for the microbiological results.

The same technique should also be suitable for hepatic pyruvate carboxylase since this enzyme is also related to dietary biotin content (Atwal et al., 1971). Using this method Anderson et al. (1978) found the biotin availability in wheat and barley to be 51 and 5 μg/kg, higher and lower values respectively than reported by Frigg (1976) and Whitehead et al. (1982). The latter explained the difference in result by the fact that blood and hepatic pyruvate carboxylase were both influenced by dietary fat and protein. To remove any confounding influence these dietary components were equalised by Whitehead et al. (1982), whereas Anderson et al. (1982) did not take this precaution. Additionally, Anderson et al. (1982) did not undertake a microbiological assay so no direct comparison is possible for the percentage availability of biotin.

The work on the biotin availability of feedstuffs has been undertaken on chicks. The assumption that the bioavailability of biotin to the pig is approximately of the same order as to the chicken appears valid following experiments by Völker et al. (1977). Even so, it is clear that the biotin content of feedstuffs is highly variable. Not only do estimates of total and available biotin content vary widely between

14

FIGURE 2

Change in intake of vitamins A, D and biotin per weaner at 8 wks expressed as a percentage of 1967 value
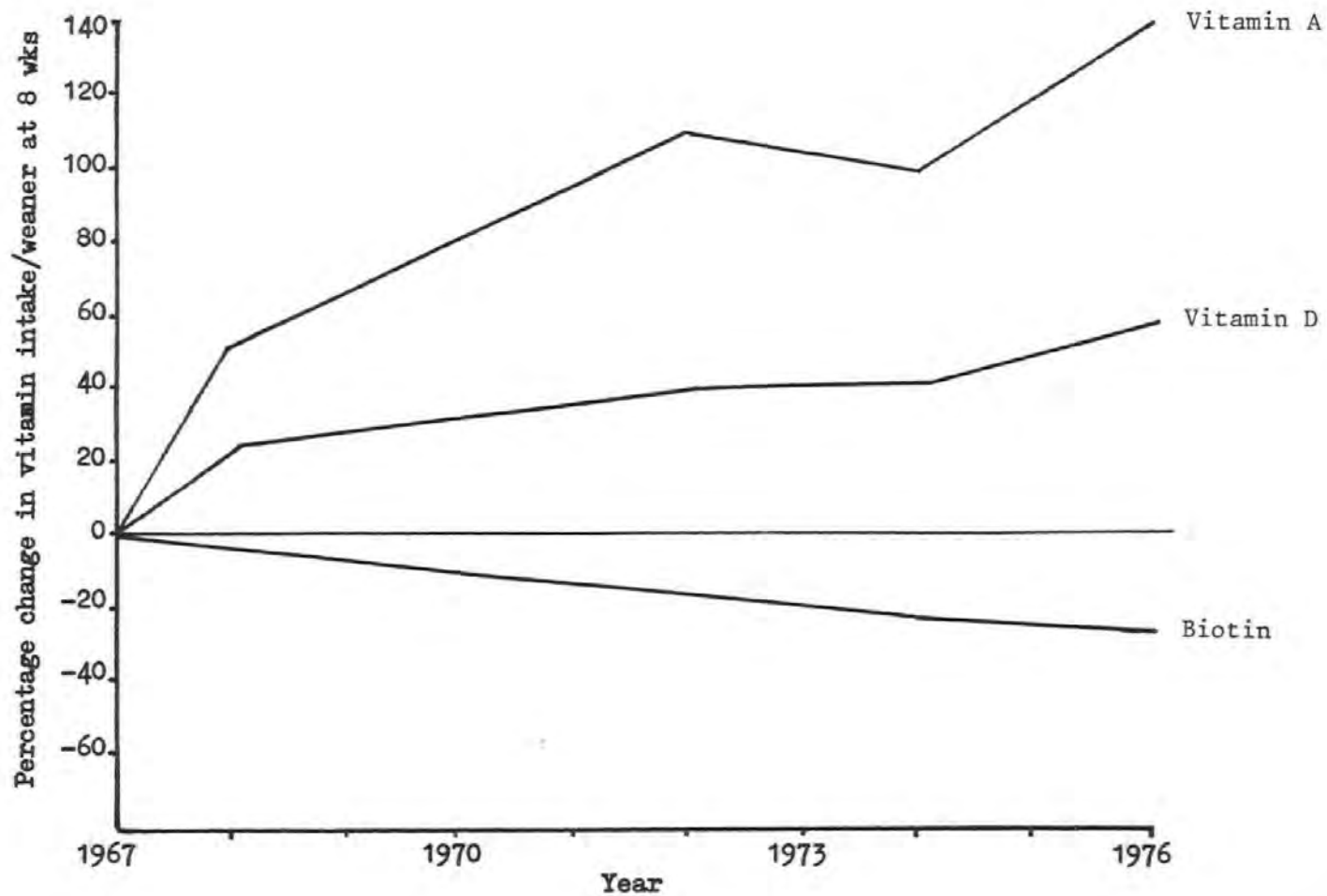
different raw materials, but also between different samples of the same raw material. This could explain why signs of biotin deficiency may develop on commercial pig units. In particular, changes in feed formulations which take no account of biotin content may result in significant changes in the provision of dietary biotin. Comben (1978) observed that, during the 1970's, as a result of changes in the relative price of cereal grains on the world markets, the proportion of use of home-grown cereals to maize had increased greatly in UK pig rations. He stated that foot lesions might be noticed about five months after a change in ration formulation which involved the exclusion, or reduction in level, of maize in favour of wheat and/or barley. Such a change would reduce the pig's biotin intake due to the reduced biological availability of biotin in the latter two feedstuffs.

The changes in diet described by Comben (1978) and in feed allowances described by Brooks (1978) reduced the amount of biotin available to the sow. Brooks (1978) calculated that the biotin provision per weaner at eight weeks probably dropped by about 27% between 1967 and 1976. Interestingly, during this same period the provision of vitamin A increased by 140% and vitamin D by 59% (Brooks and Simmins, 1981; Figure 2).

INDUCED BIOTIN DEFICIENCY

The typical physiological and pathological symptoms of biotin deficiency in pigs were first described when diets were fed which were either semi-purified with biotin omitted, or with biotin made unavailable by the inclusion of the biotin antagonist, avidin (Cunha et al., 1946; Lindley and Cunha, 1946; Lehrer et al.,1952). Their descriptions of the symptoms of biotin deficiency were confirmed and extended by Glattli et al., (1975) and Pohlenz (1974, 1975).

The first signs of biotin deficiency were poor weight gains, feed conversion and roughening of the hair coat. The animal eventually became hairless and the skin scaly. By the third week the tongue was furry and fissured and pustules were developing on the skin. Further deterioration resulted in the skin being encrusted and covered with brownish waxy layers; finally fissures formed. At this stage histological examination showed the epidermis to be irrgularly keratinised and to have a loose

structure (Pohlenz, 1975). Defects in the claw appeared at an early
stage. At first, the epidermis of the horn became eroded then
fissures or cracks formed in the horny skin of the soft heel and this
affected the deep layers of the underlying tissue. The horn became
rubbery and lesions developed at the coronet and the hard horn areas
of the side-wall and toe. The horn was now less resistant to abrasion.
Pigs kept on concrete floors not only developed large cracks along the
caudal edge of the toe, but also the soft horn of the heel was sloughed
off.

Biotin supplementation of biotin-deficient pigs has resulted in
improved weight gains (Cunha et al.,1968; Glättli, 1975). Other work
has also shown that biotin supplementation could improve weight gain
and feed conversion of pigs on commercial rations even though these pigs
had not previously shown signs of deficiency (Labuda, 1966; Zivkovic et
al.,1970; Cunha, 1971). Conditions of dry scabby skin, zinc-resistant
parakeratosis and mild exudative epidermitis in piglets, growing and
finishing pigs have also responded respectively to either a single or
few times weekly repeated injections of 1 ml biotin/10 kg body weight
(Halama, 1979; Glättli, 1975, 1976). Topical infection of the skin with
Staphylococcus hyicus caused a statistically significantly stronger
wound reaction in biotin-deficient piglets (Stuker and Glättli, 1976;
Glättli, 1976). This suggests that the skin of a biotin-deficient pig
is more susceptible to infection. It has long been established that in
the rat biotin deficiency results in diminished resistance to infection
(Caldwell and Gyorgy, 1943; Kligler et al., 1946). An impairment in
antibody response in biotin-deficient pigs has since been discovered by
Carter and Axelrod (1955), Pruzanski and Axelrod (1955) and Petrelli and
Marsili (1969). Work on the biotin-deficient rat indicates that the
healing of the wound itself is also likely to be retarded (Bosse and
Axelrod, 1948; Okey et al., 1950).

Stress may compound the effects of biotin deficiency. The fatty liver
and kidney syndrome in growing poultry can result from several factors,
including stress, combined with low dietary biotin levels (Johnson et al.,
1976; Whitehead et al.,1975). The disease is responsive to biotin
administration (Blair and Whitehead, 1974; Payne et al., 1974). Stress
has also been shown to affect biotin-deficient rats. Exposure to cold

has resulted in the death of biotin-deficient rats, although no signs of adrenal insufficiency were observed (Ratsimanga and Nigeon-du-Reuil, 1960).

Work has been undertaken in rats and chicks on the effects of biotin deficiency on reproductive function. Biotin deficiency has been induced more easily in male than in female rats (Okey et al., 1950). The same report also confirmed the influence of the sex hormones in this effect. Testosterone implants in both sexes increased the severity of a mild biotin deficiency. However gonadal hormones had little effect on the patterns of response to biotin deficiency of avian hepatic and oviducal enzyme-specific activities (Balnave, 1975; Balnave and Jackson, 1974). Unlike external symptoms, lesions of the gonads caused by biotin deficiency have not been affected by subsequent biotin administration. Lesions of the testes of male rats (Communal, 1957; Delost and Terroine, 1969) and atresia of the ovaries of female rats (Okey et al., 1950) failed to respond to supply of biotin, whereas all external symptoms of biotin deficiency disappeared.

Work has been undertaken showing the effects of induced biotin deficiency (by feeding 4% egg white in a ration) on the hoof integrity and reproductive performance of sows (Misir and Blair, 1983; Tables 6, 7, 8 and 9). The incidence of hoof cracks increased in young sows fed biotin-deficient diets but no comparison was available with sows given biotin-supplemented feed. A comparison was available for reproductive performance and an increase in litter size of 20% and 21% was observed with biotin supplementation compared with the biotin-deficient diet for first and second parity sows (Table 8). Biotin supplementation also reduced the weaning to remating interval (Table 9). The experiments of Misir and Blair (1983) were incomplete and not fully analysed. However they showed that a reduction of reproductive performance may be possible when commercial diets are fed with a low content of available biotin. The response of sows to biotin supplementation of non-synthetic diets is discussed in the following section.

A further response of an animal to induced biotin deficiency has been shown by the fatty acid metabolism. Dietary biotin supplementation of chicks has slightly increased the stearic and oleic acid levels, whereas the palmitic acid level was unaltered compared with biotin-deficient

18

diets (Roland and Edwards, 1971). There were no consistent responses shown by any of the other fatty acids studied. The ratio of sixteen carbon to eighteen carbon fatty acids was increased in biotin-deficient chicks on each diet. Except for corn-oil fed birds, the ratio of saturated to unsaturated fatty acids in biotin-deficient treatments was slightly decreased when compared to the same diet supplemented with biotin.

In biotin-deficient pigs, the lipid content of the adipose tissue was decreased and the fatty acid pattern changed, in particular, with a general increase of the mono-unsaturated acids (Bühlmann, 1973 cited by Tagwerker, 1974). The concentration of myristic was almost doubled and the concentration of its derivatives with their longer chains (palmitic and stearic) was significantly decreased (Table 5). The adipose tissue was greyish and much softer than in the control animals which resulted in undesirable carcass characteristics.

Logani et al. (1975) discovered that turkey poults on a biotin-deficient diet suffered significant alterations in the cutaneous neutral lipids of the feet. There was a reduction in total skin lipid, confined mainly to the triacylglycerols and diol diesters. The significance of these changes in lipid composition in relation to the skin lesions is uncertain; while defatting of the epidermis can reduce its water-binding capacity and its resilience, the effect of more selective alterations is poorly understood. This suggests that a knowledge of the hoof lipid composition of the pig may be important in understanding the changes in hoof structure that occur in conditions of clinical biotin deficiency.

Dermal lesions of the feet in animals fed fat-free or biotin-deficient rations have been reported in rats, mice, guinea pigs, dogs and rabbits (Ahluwalia et al., 1967; Burr and Burr, 1930; Reid, 1954; White et al., 1943) which suggests a link between biotin and essential fatty acid deficiency. Roland and Edwards (1971) cited a report by Negteren (1963) that in the transformation of arachidonic acid in the microsomes, the two carbon donor was malonate since malonyl Co A was 20 to 30 times more efficient that acetyl Co A in this chain-elongating system. Since biotin was necessary for the synthesis of malonyl CoA, this was a direct indication that biotin was involved in the conversion of linoleic to

TABLE 5[/]

The relative proportion of fatty acids in the adipose tissue of piglets

Fatty acid	Fatty acid (in % of total fatty acid content)		Change in % in deficient pigs	Signif-icance (t-test)
	Control	Biotin-deficient		
14:0 Myristic acid	1.8	3.4	+ 89	0.001
16:0 Palmitic acid	26.3	19.7	- 25	0.001
16:1 Palmitoleic acid	3.7	8.7	+ 135	0.001
17:0 Margaric acid	0.9	2.1	+ 133	0.001
18:0 Stearic acid	15.4	5.4	- 65	0.001
18:1 Oleic acid	41.9	44.5	+ 9	-
18:2 Linoleic acid	8.8	12.9	+ 47	0.001
18:3 Linolenic acid	0.5	1.1	+ 120	0.05

[/] Bühlmann, (1973)

arachidonic acid. It had been reported that arachidonic is three to four times as effective as linoleic acid in preventing essential fatty acid deficiency. Roland and Edwards (1971) failed to shew a direct link between biotin deficiency and polyunsaturated fats in the chick carcass. The addition of arachidonic acid failed to improve the dermal lesions of the chick. These results have been supported by Whitehead et al.(1976) who found that adding unsaturated fat or protein to a diet containing low levels of fat, protein and biotin resulted in reductions of growth rate and increases in the severity of lesions.

Interestingly, the fatty acid content of sow's milk may also be expected to be influenced by the biotin status of the pig. The condensation reactions of acetyl Co A in the mammary gland which synthesise palmitic and shorter chain acids (Tollerz and Lindberg, 1965) are biotin-mediated. However the output of fatty acids in milk is also influenced by the intake of dietary fat (Witter and Rook, 1970). They further observed that the milk fat composition reflects the changes in composition of plasma triglycerides.Should the level of palmitic acid in the plasma triglycerides increase, there is an associated increased output in milk triglycerides of not only palmitic acid but also palmitoleic acid due to a desaturase system.

In fact, dietary fatty acids affect the composition of the fat deposited both in terms of digestibility and metabolism of fatty acids. The levels in the plasma triglycerides of the major acids, palmitic, stearic and oleic, may not be especially sensitive to changes in uptake from the gut, but the uptake of longer-chain saturated acids may be limited by digestibility especially when present as simple triglycerides (Witter and Rook, 1970). The longer-chain fatty acids are mainly incorporated into triglycerides and transported by the lymph in the form of chylomicra, whereas the shorter-chain fatty acids are absorbed into the portal blood and transported as free fatty acid-albumin complex to the liver where they are rapidly oxidised (Senior, 1964). When feeds containing fatty acids such as lauric acid, usually absent from deposits, are introduced into the diet, these are almost entirely oxidised (Rerat, 1972). In contrast, Rerat (1972) pointed out, the pig stores about one half of the linoleic it consumes; the level of palmitic acid is almost constant (25%) whatever its proportion in the feed, whereas the level of stearic acid fluctuates considerably. The concentration of oleic acid in the fat

21

deposited is almost the same as that in the mixed feed consumed and Rerat (1972) observed that the replacement of oleic acid by saturated fat could reduce the level of "soft" fat in an animal as the former accumulates in body fat at higher levels than those present in the feed.

The influence of dietary fat on body fat highlights the importance of ensuring that the symptoms described may be proven to be as a result of biotin deficiency and not some other factor. This may be achieved where biotin deficiency is induced by a synthetic ration or the inclusion of a biotin antagonist, but great care must be taken to ensure that symptoms are due to inadequate dietary biotin when animals are fed non-synthetic diets.

RESPONSES OF SOWS TO BIOTIN SUPPLEMENTATION OF NON-SYNTHETIC DIETS

Introduction

Supplementation of commercial sow diets with biotin has been thought unnecessary as it was previously considered that sufficient dietary biotin was available from commercial feeds (ARC, 1967). Sufficient information is still not available to provide an estimate of requirements for biotin in pigs. Therefore diets have been formulated without reference to the biotin content. However, evidence presented in the section on "bioavailability of biotin in feedstuffs" suggests that low levels of available biotin may be provided by some raw materials. Consequently diets formulated to meet other nutrient requirements without reference to their biotin content may have widely differing biotin levels depending on the raw materials used in formulation. There is evidence to suggest that some diets may not provide sufficient biotin to satisfy the pig's requirements. Symptoms similar to those of clinical biotin deficiency have been reported in herds given commercial feeds and in a number of cases these have been responsive to biotin supplementation. In addition, sows have also responded to biotin supplementation when no previous signs of clinical biotin deficiency have been observed. The response of the sow to biotin supplementation has been in two areas:-

improvement of hoof integrity; and
improvement of reproductive performance.

Hoof integrity

Between 8 and 10% of all culling is for lameness and leg weakness
(PIDA, 1964; Einarrson and Settergren, 1974; Svendsen et al., 1975;
Dagorn and Aumaitre, 1979). The report by PIDA (1964) showed that the
majority of culling for lameness in breeding stock occurred in first or
second litter sows, but those sows culled were only a small proportion
of the total stock with foot lesions. That the presence of foot lesions
does not necessarily result in culling was substantiated by Penny et al.
(1963) who noted that 65% of bacon weight pigs had foot lesions.
Clearly the severity of damage to the hoof is significant. In the out-
break of lameness in sows observed by Brooks et al.(1977) on a commer-
cial unit, the level of foot lesions was very high and equivalent to
the level reported by Penny et al.(1965) for sows recently moved to pens
with rough concrete from a free-range system. However the herd studied
by Brooks et al.(1977) had not had a change of flooring. Nevertheless
the flooring can influence the degree of injury caused to the horn of a
pig's hoof. Grandhi and Strain (1980) showed that gilts raised on dirt
lots had significantly more lesions than those reared on concrete. The
types of lesion observed were similar on both floors which, they
suggested, indicated that the nature of the lesions was independant of
the type of floor on which pigs were raised. Results obtained in other
species, notably cattle, suggest that the level of damage to a pig's
hoof horn would vary not only with environment but also with breed.
Although there is little evidence to support this supposition to date,
an inadequate biotin supply may influence the resistance of claws to
damage, rather than, as it was thought previously, itself producing
lesions. Tagwerker (1973), in describing the trials of Bühlmann (1973),
Bühlmann et al.(1973) and Glättli et al.(1975), observed that on a
biotin-deficient diet, the hoof horn of the pig became rubbery compared
to that of pigs on a biotin-supplemented diet. Comben (1978) also
observed softness of hooves in commercial herds with a high incidence of
lameness and foot lesions which responded to high intakes of dietary
biotin. More recently, Whitehead et al.(1980) noted that young pigs
developed a pronounced softness of the heel pad when fed diets with
little or no available biotin. None of the pigs in this last trial
developed lesions which, the authors considered, was because they were
housed on smooth metal floors rather than a more abrasive surface, such
as concrete. Thus the biotin status of a pig may influence the physical

properties of a hoof such that on a biotin-deficient diet the pig's hoof is rendered more susceptible to physical damage. Cunha et al. (1968) were the first to report biotin deficiency in pigs kept under farm conditions in North and South America. Field cases from the Far East and Europe of ailments accompanied by skin or claw lesions, or both, have since been recorded (Tagwerker, 1973; Comben, 1978).

Success in reversing skin and claw lesions similar to those produced by experimentally induced biotin deficiency has been achieved only when high doses of biotin were given, over and above the requirements estimated by Marks (1975). Glättli (1975) reported that spontaneous claw lesions in sows responded favourably to the administration of 5 mg biotin per sow per day for a period of 2 months. The response, although slower, was comparable with the response shown by piglets with induced biotin deficiency, following restoration of biotin supply (Tagwerker, 1973). However provision of biotin by intramuscular injection was ineffective in the experiment undertaken by Brooks et al. (1977) and less effective than a combined treatment of injections plus supplementation of biotin in the feed (Bujas et al., 1972 cited by Tagwerker, 1974; Table 7).

Neither of these trials provided injections of biotin on a daily basis (Table 6) and consequently the biotin provided would have been lower than estimated as it is a water soluble vitamin which cannot be stored if in excess of the immediate metabolic demand of the animal. Therefore a more effective response might occur as a result of providing supplemented biotin on a daily basis.

Most workers have chosen the approach of providing a daily intake of biotin via the feed. In the second experiment reported by Brooks et al. (1977), six months of dietary biotin supplementation resulted in a reduced overall incidence of lesions compared with a control group (Tables 6 and 7). In subsequent trials (Tables 6 and 7), only Michel and Mastachi (1981) failed to obtain a response to biotin supplementation on hoof horn lesions. The sows in this trial were initially healthy and were between their second and fourth parities. The significance of the latter point may be seen by examining the results of other experimenters. Bryant et al. (1982) commenced treatment with initially healthy stock,

TABLE 6

A summary of experimental procedures in trials investigating the effect
of supplementary biotin on the performance of the female pig

Authors	Main dietary ingredients	Available biotin in basal diet (μg/kg)	Level of biotin supplementation in feed (μg/kg)	Duration of trial	Trial condition
Bujas et al. cited by Tagwerker (1973)	_[1]	_[1]	i) injection of 5 mg biotin twice a week	4 weeks	All sows were suffering initially from severe hoof lesions; 5 sows/treatment.
			ii) as above plus feed supplement of 500		
Spörri (1976)	_[1]	89/90	Injection of 5 mg/ sow/day	at least 2 months	20 sows given biotin, 20 sows given placebo.
Brooks et al. (1977)	Pregnancy = barley Lactation = barley and wheat	74[2]	Experiment 1 = injection of 3 x 2 mg biotin at 5 day intervals	Exp 1 = 3 months	High level of severe hoof lesions in herd at start of trial, which did not respond to other treatments.
			Experiment 2 = pregnancy = 200 lactation = 150	Exp 2 = 6 months	Exp 1 = 10 control and 8 supplemented sows Exp 2 = 27 sows/treatment
Easter et al.(1979)	Maize, soya	_[1]	200	Gestation	20/22 gilts on each treatment; initially healthy.
Halama (1979)	_[1]	74	Pregnancy = 90 Lactation = 120	_[3]	Herd suffered from skin disorders prior to experimentation. Whole herd (170 sows) given supplemented biotin; comparison with pre-treatment performance.
Triebel and Lobsiger (1979)	Barley, oats, wheatings, maize	55	500	4 months	25 control and 17 supplemented young sows.
Money and Laughton (1980)	Barley	38	Pregnancy = 290 Lactation = 329	4 months	6 control and 13 supplemented sows.
Grandhi and Strain (1980)	Barley, wheat, soya	Pregnancy = 93 Lactation = 104	200/300[4]	4 months/ trial	237 first and second parity sows[4].
Newman and Elliot (1980)	Barley, soya	_[1]	_[1]	_[1]	First parity sows[5].
Pedersen and Udesen (1980)	Barley, soya	29-64	i) First 5 weeks = 400 ii) 3 months = 2000 iii) 3 months = 400	Over 7 months	Initial high level of hoof lesions on commercial herd of 140 sows; all sows and gilts given either control or supplemented diet.
Penny et al. (1980, 1981)	_[1]	56	Pregnancy = 1160 μg/day[6] Lactation = 2320 μg/day	12 months	Initial high level of hoof lesions & lameness on commercial herd of 116 sows; all sows and gilts allocated to control and supplemented treatments on basis of parity, liveweight and number and severity of foot lesions. Replacement gilts allocated alternatively to either treatment.
Michel and Mastachi (1981)	Sorghum	74	Two supplemented treatments = 100 or 200	6 months	30 initially healthy sows, between 2 and 4 parities. Biotin supplementation provided between day 30 and 109 of gestation.

TABLE 6 (contd.)

Authors	Main dietary ingredients	Available biotin in basal diet (µg/kg)	Level of biotin supplementation in feed (µg/kg)	Duration of trial	Trial condition
Robres Serrano and Garcia de la Calera (1981)	Barley, wheatings, soya	103– 155	200	Exp 1 = 574 days Exp 2 = 673 days	Exp 1 = initially healthy sows of various parities. Results from farrowings of 145 and 138 control and biotin supplemented sows (herd of 120 sows). Exp 2 = 49 daughters of the Exp 1 biotin supplemented sows were fed either the control or biotin supplemented diets.
Bryant et al.(1982)	2 diets: 1) Maize, soya 2) Wheat	1) 123^7 2) 152^7	1) 220 2) 440	4 parities	116 gilts were allocated to either a control or biotin-supplemented treatment of one of two diets. Gilts were allocated at 100 kg liveweight. Prior to treatment gilts had been given a biotin-supplemented diet.
De Jong and Sytsema (1983)	Manioc, maize gluten, soya, maize, wheat	100	Gilts – 1250 Sows – 500	Young stock 4 months Gilts – 2.5 months Sows – 11 months	Culling rate due to lameness very high prior to treatment; whole herd treated (130 sows); comparison with pre-treatment performance.
Hamilton et al.(1983)	Maize, soya	170^8	500	3 years	Whole herd (161 sows) utilised[9].
Tribble (1983)	Sorghum, soya	Pregnancy = 103 Lactation = 107	200	4 parities	53 gilts were allocated to either a control or biotin-supplemented treatment following mating.
Misir and Blair (1983)	Barley, wheat	135^{10}	250^{11}	In progress (12 months)	16 sows on each treatment.

[1]Information not presented.

[2]Estimated level of available biotin; total biotin was assayed as 124.7 and 117.6 µg/kg for Diet A and 99.2 and 108.6 µg/kg for Diet B for trials 1 and 2 respectively.

[3]Information not stated; at least one year.

[4]Two experiments, the results were combined.

[5]Animals assumed to be initially healthy; numbers were not available.

[6]Additional biotin provided in the form of a supplement, which contained 29 mg biotin/kg, of 40 g/day during pregnancy and 80 g/day during lactation.

[7]Biotin levels as determined by microbiological assay.

[8]Incomplete information was presented on the diet. It is assumed that total biotin content of diet was given.

[9]No information was presented on condition of sows prior to treatment and on age structure of herd.

[10]Data available for total biotin content, 4% egg white was added to control diet which was considered to contain sufficient avidin to bind all available biotin.

[11]Biotin-supplemented diet excluded egg white.

TABLE 7

Effects of dietary biotin supplementation on hoof integrity of the sow

Author	Response
Bujas et al. cited by Tagwerker (1974)	Control treatment: no reduction in degree of severity of lesions; Injection only: some reduction in severity; Injection plus feed supplementation: almost completely healed lesions.
Sporri (1976)	Positive effect of biotin supplementation, but doses above 5 mg/sow/day not likely to improve results.
Brooks et al. (1977)	Experiment 1: no effect on hoof lesions. Experiment 2: biotin supplementation reduced overall incidence of lesions by 28%, no effect on control group; difference mainly in toe and soft heel.
Triebel and Lobsiger (1979)	Following treatment: claw cracks (41.9% vs 7.5% for control and supplemented pigs respectively) and erosions (22.1% vs 2.5% for control and supplemented pigs respectively).
Money and Laughton (1980)	Reduced number of foot lesions (12.6 and 10.3 versus 11.8 and 2.8 at beginning and completion of the trial for the control and supplemented groups respectively).
Grandhi and Strain (1980)	The lesion scores were not significantly different between treatments but the magnitude of increase from the initial to the final lesion score was less in the supplemented group.
Pedersen and Udesen (1980)	Reduced number of claw lesions from 21.8/sow before treatment to 13.0/sow.
Penny et al. (1980)	No improvement in severity and number of lesions/sow except for replacement gilts.
Michel and Mastachi (1981)	No effect.
Bryant et al. (1982)	Reduced hoof lesions mainly after first parity.
De Jong and Sytsema (1983)	Young stock – greater increase in lesions in control group (15.0 vs 7.2 for control and supplemented sows respectively). Gilts – lesions reduced by 28% in supplemented sows, no effect on control sows. Sows – culling rate due to lameness reduced from 25% (in the previous year) to 14%.
Misir and Blair (1983)	Increased incidence of hoof cracks over 12 months[1].

NOTES

[1] No comparison with supplemented sows.

Easter et al (1979), Halama (1979), Newman and Elliot (1980), Robres Serrano and Garcia de la Calera (1981), Hamilton et al (1983) and Tribble (1983) did not investigate hoof integrity.

which were fed a biotin-supplemented diet prior to allocation to treatment. The response to biotin supplementation was not marked in the gilts in the three trials reported by Bryant et al. (1982), but in one trial, biotin supplementation resulted in significantly less heel crack lesions. Penny et al. (1980) observed no effect of biotin supplementation on the severity and number of hoof lesions per sow in a herd which had a high level of hoof lameness prior to treatment. However, the replacement gilts introduced into this herd, which were assumed to be healthy initially, did have significantly fewer and less severe lesions in seven out of forty-four categories when examined at first weaning. Penny et al. (1980) concluded that although established lesions did not appear to respond to biotin supplementation at the levels used, the feet of young gilts with few foot lesions on entry to the herd were afforded some protection by biotin supplementation. De Jong and Sytsema (1982) also concluded from their trials that biotin supplementation reduced the rate of development of claw lesions. Although lesions increased rapidly in gilts between five and seven months of age in both biotin-supplemented and unsupplemented treatments, the biotin-supplemented group had significantly ($p < 0.001$) fewer lesions than the controls. This response was repeated in a trial they undertook with older gilts. Similarly the work reported by Grandhi and Strain (1980) showed that, for first and second parity sows, although the number of lesions increased in both biotin-supplemented and unsupplemented sows, the rate of increase was less with biotin supplementation. Therefore the lack of response reported by Michel and Mastachi (1981) would suggest that biotin supplementation may have less effect on the hoof horn of older stock which are considered to be initially healthy. However, responses to dietary biotin supplementation are possible in older sows if the initial incidence of hoof lesions is high. (Brooks et al., 1977; Triebel and Lobsiger, 1979; Money and Laughton, 1980; Pedersen and Udesen, 1980; De Jong and Sytsema, 1983). In all these trials a reduction in the number of lesions was obtained as a result of dietary biotin supplementation, although an improvement in hoof condition and abnormal leg positions was reported by Pedersen and Udesen (1980) in the control sows as well. This may be explained by an improvement in biotin availability of the basal diet as the plasma biotin levels of the control animals also increased in the latter part of the

27

trial. Nonetheless, the biotin-supplemented sows still had a sig-
nificantly (p ⩽ 0.05) lower incidence of claw lesions than the control
sows at the completion of the trial.

The improvement in hoof health following dietary biotin supplementation
has also produced an improvement in the productivity of herds. De Jong
and Sytsema (1982) reported a reduction in the culling rate due to lame-
ness from 25% to 14% and the culling rate as a result of "insufficient
production" from 11% to 4%. The latter result may be explained not
only by improved hoof health but also by the effect that biotin supp-
lementation has on reproductive performance which may be unrelated to
any effects due to lameness.

Reproductive performance
Observations by Cunha (1971) on the effect of biotin supplementation on
reproductive performance were first confirmed by Brooks et al. (1977)
who showed that biotin supplementation produced a significant increase
in liveborn pigs in second parity sows and a significant reduction in
the length of the weaning to remating interval (Tables 8 and 9). Comben
(1978) has also reported similar results from field cases.

Most of the trials undertaken subsequently have confirmed that biotin
supplementation improves litter size and reduces the weaning to
remating interval (Tables 8 and 9). As may be expected the weaning to
remating interval was longest following the first lactation compared to
later lactations (Penny et al., 1981; Bryant et al., 1982; Tribble, 1983).
Biotin supplementation was most effective in reducing this interval
following the weaning of the first and second litters (Penny et al.,
1981) and the first litter (Tribble, 1983). These results further
suggest that biotin supplementation is most effective at improving the
performance of the young sow. Therefore it may explain why no difference
in the weaning to service period was observed when results for sows with
a wide age range were amalgamated (Hilton et al., 1983). Pedersen and
Udesen (1980), who reported a shorter interval between weaning and con-
ception, felt that part of the treatment differences could be explained
by fewer returns to service in the biotin-supplemented group.
Interestingly, Bryant et al. (1982) showed not only a response to biotin
supplementation, but also between the two basal diets fed. Sows fed a

28

TABLE 8

Effects of dietary biotin supplementation on postweaning performance of the sow

Author	Weaning to remating interval without biotin supplementation (days)	Weaning to remating interval with biotin supplementation (days)	Weaning to effective service without biotin supplementation (days)	Weaning to effective service with biotin supplementation (days)	Notes[1]
Brooks et al. (1977)	15.31 ± 2.85	6.23 ± 2.85	-	-	Statistically significant difference (p < 0.05)
Halana (1979)	8-10	6-9	-	-	Comparison with previous year's data
Grandhi and Strain (1980)	7.62	7.72	-	-	
Newman and Elliot (1980)	-	-			No treatment effects. (Data not presented)
Pedersen and Udesen (1980)	10.8	8.9	37.2	26.2	
Penny et al. (1981)	18.67	13.80	-	-	Following first weaning
	5.33	3.00	-	-	Following second weaning (statistically significant difference, p < 0.05)
	5.75	15.67	-	-	Following third weaning
Robres Serrano and Garcia de la Calera (1981)	-	-	15.24	14.98	
Bryant et al. (1982)	14.5	10.2	-	-	Statistically significant difference (p < 0.05)[2]
Hamilton et al. (1983)	6.98	6.71	-	-	
Tribble (1983)	14.72	12.26	-	-	Following first weaning
	5.58	7.92	-	-	Following second weaning
	4.95	5.32	-	-	Following third weaning
	5.28	5.15	-	-	Following fourth weaning
Misir and Blair (1983)	8.10 ± 2.26[3]	5.11 ± 0.20	-	-	Following first weaning
	11.00 ± 3.40[4]	4.67 ± 0.05	-	-	Following second weaning

[1]Differences between treatments not statistically significant unless otherwise stated.

[2]Parity data amalgamated.

[3]Includes data from 10 control sows, 6 other control sows gave interval of 126.2 ± 21.0.

[4]Includes data from 10 control sows, 1 other control sow gave interval of 58 days.

Bujas et al. cited by Tagwerker (1973), Spörri (1976), Easter et al.(1979), Triebel and Lobsiger (1979), Money and Laughton (1980), Michel and Mastachi (1981), De Jong and Systema (1983) did not investigate postweaning performance.

TABLE 9

Effect of biotin supplementation on litter size in the sow

Author	Mean no. of piglets born alive/litter without biotin supplementation	Mean no. of piglets born alive/litter with biotin supplementation	%age improvement in performance	Notes[1]
Brooks et al. (1977)	8.13	9.77[1]	16.8	Second parity (statistically significant difference, p <0.05)
	10.89	10.30[2]	- 5.7	Third parity (3.1% more total pigs born to supplemented sows)
Easter et al. (1979)	8.5	9.2	7.6	First parity
Grandhi and Strain (1980)	9.18	8.97	- 2.3	
Newman and Elliot (1980)	-	-	-	No treatment effect. (Data not presented)
Pedersen and Udesen (1980)	9.6	10.0	4.0	
Penny et al. (1981)	11.00	8.25	- 33.3	First parity
	8.83	12.00	26.4	Second parity (statistically significant difference, p <0.05)
	8.00	11.44	30.1	Third parity
	9.33	11.77	20.7	Fourth parity (statistically significant difference, p <0.05)
				Parities 5 to 7 had low sample numbers and showed no treatment differences
Michel and Mastachi (1981)	8.00	8.14	1.7	100 µg supplement (statistically significant difference, p <0.05)
		8.44	5.2	200 µg supplement (statistically significant difference, p <0.05)
Robres Serrano and Garcia de la Calera (1981)	9.45	9.85	4.1	
Bryant et al. (1982)	10.09	10.35	2.5	Parity data amalgamated
De Jong and Sytsema (1983)	12.57	11.84	(- 6.2)	Total pigs born; comparison with previous year's data
Hamilton et al (1983)	9.24	9.08	- 1.8	
Tribble (1983)	8.15	7.96	- 2.3	First parity
	7.96	8.84	10.0	Second parity
	9.96	10.45	4.7	Third parity
	9.50	11.23	15.4	Fourth parity (statistically significant difference, p <0.05)
Misir and Blair (1983)	7.88 ± 0.80	9.81 ± 0.36	9.7	First parity
	8.15 ± 0.04	10.27 ± 0.04	20.6	Second parity (Further statistical analysis not undertaken)

[1] Differences between treatments not statistically significant unless stated otherwise.

Bujas et al. cited by Tagwerker (1973), Spörri (1976), Halama (1979), Triebel and Lobsiger (1979), Money and Laughton (1980) did not investigate litter performance.

maize-soya diet showed an improvement in days to oestrus and conception
rate compared with those fed a wheat-based diet which may also be due
to a difference in the biotin available to the sow. The available biotin
content of the wheat-based diet (estimated to be 45 µg biotin/kg) would
have been considerably less than that of the maize-soya diet (estimated
to be 99 µg biotin/kg).

Biotin supplementation appeared to be most effective in improving the
number of live pigs produced per litter following the first litter.
The litter performance of first parity animals in the trials of Penny
et al.(1981) and Tribble (1983) was not improved by biotin supplemen-
tation. However significantly more live pigs were born, to biotin
supplemented sows, in the second and fourth parities, and a non-
significant improvement was obtained in the third parity (Penny et al.
1981). A similar trend was reported by Tribble (1983). In only three
trials has no benefit been demonstrated (Grandhi and Strain, 1980; De
Jong and Systema, 1983 and Hamilton et al., 1983). Grandhi and Strain
(1980) only provided biotin supplementation for four months. This may
have been an insufficient period to have an effect on reproductive per-
formance, particularly as the pigs used were not considered to be
biotin-deficient at the start of the trial. De Jong and Sytsema (1983)
used the previous year's data as comparison, hence a direct comparison
of results is necessarily limited and may have been confounded by
differences in the age profile of the herd.

Robres Serrano and Garcia de la Calera (1981) observed that the number
of dead pigs born was reduced by 9.2% and that more live pigs survived
twenty-four hours after birth as a result of biotin supplementation. A
follow-up study was carried out on the daughters of the biotin-
supplemented and control sows described above and fed the same diets as
their mothers. Unlike the mothers, the trial with the daughters reported
no significant differences in treatments. Easter et al.(1979) reported
a 5% reduction in dead pigs born to supplemented gilts, whilst Pedersen
and Udesen (1980) reported a reduction in pre-weaning piglet mortality
in biotin-supplemented sows. Dietary biotin supplementation has also
increased litter weight compared to control sows (Michel and Mastachi,
1980; Tribble, 1983).

The trials reported above were of a relatively short duration involving either first and second parity animals only or animals of mixed age studied over one or two reproductive cyles. Exceptions were the longer-term investigations undertaken by Bryant et al. (1982) and Tribble (1983). However the gilts did not commence treatment in the experiments of Bryant et al. (1982) until they were 100 kg liveweight, prior to which they were fed a biotin-supplemented diet. This feeding regime may have confounded the effect of a biotin-unsupplemented diet on the hoof integrity and reproductive performance of the young sow. Tribble (1983) fed a sorghum-soya diet which has a high available biotin content and is not a typical UK diet.

MEASUREMENT OF THE BIOTIN STATUS OF THE PIG

Clearly an inadequate provision of biotin has a complex effect on the physiology, pathology and biochemistry of a pig. Reproduction, itself a complex metabolic process, would be affected by the varied effects of low biotin levels discussed earlier. A judgment of whether the biotin status of a commercial animal is a constraint on its performance would depend upon the availability of an acceptable measure of biotin status in the live animal.

A number of workers have used plasma biotin levels as an indicator of dietary supply and/or biotin status. Tagwerker (1973) reported that biotin-deficient piglets had plasma biotin concentrations of twenty to thirty nanograms per one hundred millilitres (ng/100 ml) plasma compared with 40 ng/100 ml in normal piglets. However the technique is limited in several important aspects. Biotin, being water soluble, is not stored in an animal's tissues so that intermittent orally or parenterally administered biotin gives a transient elevation of blood levels. Surplus biotin is partly oxidised in the liver, as shown in a study on rats (McCormick, 1975), and is excreted in the urine within a few hours. However a return to initial levels may take one or two days (Baker et al., 1969; Glättli et al., 1975). It may be expected that biotin consumed in the feed would be transferred more slowly to the blood system of a pig and therefore maintain a higher plasma level for a longer period than following an injection. This may explain in part, why a short-term treatment of sows injected with biotin, did not result in any observable effects on the state of the claw lesions, whereas

32

regular provision of biotin in the feed reduced the damage to claws (Brooks et al., 1977). However in once or twice a day feeding, as is usual in sows, diurnal variation and post-prandial peaks are still likely to occur. The technique would be more reliable if the animals were ad libitum fed as is often undertaken with growing pigs and chicks.

Increasing the dietary supply of biotin results in an initial increase in plasma biotin concentration but sustained supplementation resulted in lower values (Völker et al., 1977, cited by Tagwerker, 1977). The authors discovered that supplementation up to 100 µg/kg (total biotin intake of 190 µg/kg) of feed achieved relatively low plasma levels which decreased with the progress of the experiment. Symptoms of clinical deficiency were observed in groups showing average plasma levels of less than 50 ng/100 ml. Higher supplements produced increases up to almost 2900 ng/100 ml, but again lower values in the final weeks of the experiments. It was suggested by Tagwerker (1974) that this indicated an equilibrium state had developed after several weeks of intensive biotin intake.

As most work on plasma biotin levels has been undertaken on young pigs in experimental conditions, only small differences have been observed in levels between the states of biotin deficiency and sufficiency (Tagwerker, 1973). Tagwerker (1977) also considered that the information on blood plasma levels in older pigs was too rudimentary to permit a reliable correlation with states of normality, various degrees of deficiency or responsiveness to biotin supplementation. Furthermore the biological variation between individual animals in apparently healthy stock is likely to be considerable, effectively reducing the technique to a method of providing only mean population values and direct comparisons between treatments under experimental conditions. Consequently other techniques have been examined to discover whether they may provide a more accurate assessment of the biotin status of the individual pig.

The measurement of biotin-dependent enzymes in blood plasma has been used to assess the biotin status of the bird. Pyruvate carboxylase and acetyl Co A carboxylase have been investigated. The latter is less sensitive than the former and is affected by other dietary constituents

so it is not possible to specify actual enzyme activity associated with deficiency of the vitamin (Whitehead, 1977). The acetyl Co A carboxylase occurs in the cytoplasm, whereas the pyruvate carboxylase is in the mitochondrion and its sensitivity is possibly due to its more restricted access to the available biotin. As a direct assay it has been proved suitable for chicks (Bannister and Whitehead, 1976; Whitehead and Bannister, 1978, 1980). High levels of activity occur in the blood of young broilers because avian red blood cells are nucleated and are believed to contain remnants of mitochondria (Harris, 1971). In contrast, activity in pigs is lower since the enzyme is probably confined to white cells and possibly reticulocytes. Glatzle (1979) and Whitehead et al. (1980) confirmed the lower activity, which was by a factor of more than one hundred when compared to broilers. Whitehead et al. (1980) showed a relationship between blood pyruvate carboxylase and biotin status, but the low level of activity and rapid changes with age suggested that it may not be a suitable criterion of the biotin status of the pig. Hepatic pyruvate carboxylase provides a better indication of biotin levels in mammals. Hood et al. (1976) reported that concentrations of biotin in the liver of rats varied with biotin content of the diet. The disadvantage is that the animal is usually sacrificed to obtain a liver sample.

The use of tissue fatty acid composition as a measure of dietary biotin level has been studied in the chick. Biotin deficiency has resulted in characteristic changes in liver and adipose tissue fatty acids (Roland and Edwards, 1971). Generally, there was an increase in mono-unsaturated fatty acids, notably palmitoleic acid and a decrease in the saturated fatty acids. Subsequently Whitehead (1977) in a review noted that the ratio of palmitoleic to stearic acid decreased as the dietary biotin level increased and that the measurement of this ratio in the toes of chicks was a possible way of assessing the biotin status of a flock. Whitehead (1977) also stated that it had been shown that the proportions of palmitoleic and stearic acids in liver and adipose tissue were unaffected by the dietary biotin level when the dietary levels of fat or protein were high. It may be that changes in tissue fatty acid ratios are not dependent upon biotin status alone in the chick. Bühlmann, 1973, cited by Tagwerker, 1974, showed that the level of biotin had a significant effect on the fatty acid composition

of a pig. It appears likely that this occurs when fat deposition comes from <u>de</u> <u>nova</u> synthesis but it is not clear how sensitive the changes in fatty acid composition are to the biotin status of a pig under these conditions. Further investigation of this technique could provide confirmation of the level of plasma biotin considered by Tagwerker (1973) to be indicative of biotin deficiency and may lead to greater understanding of the biotin status of the pig.

CONCLUSION

The complex role that biotin plays in the metabolic pathways indicates that the performance of an animal is likely to be influenced by its biotin status. The effects of deficiency are still not understood and further evidence is needed of the physiological and biochemical changes that occur and the role they play in the health of the animal. This may lead to the development of a reliable measure of the biotin status of the individual pig. The need for this is evident as the use of commercial diets not supplemented with biotin can result in clinical symptoms on commercial units, similar to those resulting from induced biotin deficiency, which are responsive to biotin supplementation. Furthermore there is also a need to determine whether healthy animals fed commercial diets containing low levels of biologically available biotin would benefit from biotin supplementation.

EXPERIMENT ONE
THE EFFECTS OF SUPPLEMENTING SOW DIETS WITH BIOTIN

INTRODUCTION

Maize has one hundred per cent of the biotin content available for utilisation by the pig. The availability of biotin in wheat and barley is only twenty to thirty per cent or less (Anderson and Warnick, 1970; Frigg, 1976). Comben (1978) suggested that the use of wheat and/or barley in feed formulation in preference to maize has led to cases of biotin deficiency in sows.

Investigations into an outbreak of lameness and alopecia in breeding sows revealed symptoms which corresponded with pigs experimentally-fed biotin-deficient diets (Brooks et al., 1977). Dietary supplementation of the cereal-based diet with biotin lowered the incidence of lesions in the hooves and unexpectedly improved the reproductive performance. However, the results were treated with caution due to the experimental design. The spontaneous deficiency was assumed to have afflicted all the sows prior to dietary supplementation with biotin, the treatment subsequently starting at different stages of the reproductive cycle. This phenomenon has not been corroborated under controlled conditions using initially healthy stock.

The object of the experiment reported here was to determine whether biotin supplementation of diets formulated from commonly-used raw materials low in available biotin content and fed according to current commercial practice would influence the reproductive performance of the female pig over a number of parities. The experiment had the further objectives of monitoring the hoof integrity of the sow by studying the development of hoof lesions and investigating the influence of biotin supplementation on the incidence of lesions.

36

Treatments

Eighty gilts, comprising forty pairs of full sibs, out of either Landrace x Large White or Large White sows mated to Landrace boars, were allocated at twenty-five kilogram live-weight to either:

    i.   a negative control diet; or

    ii.   a control diet plus 350 µg/kg supplementary biotin.

The sows remained on their respective treatments until weaned from their fourth litter.

Diets

A balanced diet was formulated based on wheat and barley with white fish meal as a source of protein and essential amino-acids (Table 10). All three feedstuffs had a low available biotin content (no more than 30%) so that for the two basal diets, only 31.1 µg/kg and 31.5 ug/kg biotin were available (Diets A and B respectively) whereas Marks (1975) suggested that the requirement for breeding sows should be 220 µg/kg biotin.

The diets were calculated to provide 13.1% crude protein (CP) and 13.15 megajoules of digestible energy per kilogram (MJ DE/kg) for Diet A and 14.4% CP and 13.11 MJ DE/kg for Diet B (Table 11).

The mineral and vitamin levels in the supplements were generally higher than the ARC (1967) recommended levels so that confounding effects due to other nutritional factors would be minimised (Table 11). The micro-nutrients were premixed as follows:

    i.   Vitamin mix, without choline and no added biotin for the control diets;

    ii.   Vitamin mix, without choline plus added biotin to supply 350 µg biotin/kg of complete feed for the supplemented diets;

    iii.   Choline chloride, diluted on a suitable carrier, designed to supply 300 g/t complete feed;

    iv.   Trace elements mixed together with calcium, phosphorus and salt.

37

TABLE 10

Assumed composition of feedstuffs used in the diets

Feedstuff	Oil	Protein	Fibre	Lysine	Threo	M+C	Calcium	Phosphorus	Salt	DE	Total Biotin	Availability
	%	%	%	%	%	%	%	%	%	MJ/kg	µg/kg	%
Barley	2.0	10.0	5.0	0.36	0.32	0.35	0.10	0.40	0.20	13.26	140	30
Wheat	2.0	11.0	3.0	0.34	0.28	0.38	0.05	0.35	0.10	13.63	100	20
Fishmeal	4.0	65.0	-	5.00	2.75	2.50	6.20	3.00	1.50	12.15	200	30

Feedstuff	Diet A %	Diet B %
Barley	50.0	50.0
Wheat	43.0	40.5
Fishmeal	5.0	7.5
Supplement	2.0	2.0

TABLE 11

Calculated analyses of diet A and diet B

	Diet A %	Diet B %
OIL	2.1	2.1
PROTEIN	13.1	14.4
FIBRE	3.6	3.5
Lysine	0.58	0.69
Methionine and Cystine	0.47	0.52
Threonine	0.42	0.48
Calcium	0.92	1.07
Phosphorus	0.57	0.64
Salt	0.48	0.51
Total biotin	120.2 µg/kg	121.7 µg/kg
Available biotin	31.1 µg/kg	31.5 µg/kg
Digestible Energy	13.15 MJ/kg	13.11 MJ/kg

*Mineral/vitamin supplement provided per kg diet:

Vit A, 15,000 iu; Vit D3, 2000 iu; Vit E, 20 iu; Vit K, 4 mg; Vit B1, 2 mg; Vit B2, 5 mg; Vit B6, 4 mg; Vit B12, 15 µg; Nicotinic acid, 18 mg; Pantothenic acid, 15 mg; Folic acid, 1 mg; Choline, 300 mg; Fe, 100 ppm; Co, 1.5 ppm; Mn, 50 ppm; Cu, 10 ppm; Zn, 80 ppm; I_2, 3 ppm; Se, 0.1 ppm. Plus Calcium, Phosphorus and Salt to give analysis above.

The trace elements were premixed separately from the vitamins in order to avoid the degradative effects of the heavy metals on the vitamins. The choline chloride was also stored separately as it is highly deliquescent and could cause rapid loss of activity of vitamins during storage.

The cereal grains were hammermilled and thoroughly mixed with the fishmeal, vitamin premix, mineral supplement and choline premix in half-tonne batches. Diets were mixed on a regular basis (usually weekly) to minimise the storage period for the finished diet and consequent loss of activity of any of the micro-nutrients.

Feeding

On allocation the gilts were fed ad lib. on a cubed rearing diet. At twenty-five kilogram liveweight the gilts were transferred to Diet B and individually fed once daily in the morning, according to the ration scale in Table 12. The gilts were weighed on the Monday of each week and their rations changed the following Wednesday.

Both diets were fed as a wet meal. The results from the experiment described in appendix 1 showed that no confounding effects on treatment would occur due to the diet fed. Consequently Diet A was fed from twenty-four hours following service until seven days prior to farrowing. Diet B was given from day one-hundred-and-eight of pregnancy until twenty-four hours after mating at the post-weaning oestrus. Pregnancy allocations were determined by parity number and lactation allowances were related to litter size (Table 12).

Housing and Service Management

The gilts were housed in rearing accommodation in pens of eight animals comprising four pairs of similar age and weight. The accommodation provided a solid concrete flooring with a lightly strawed sleeping area, a separate dunging passage and individual feeding stalls. The gilts were isolated from mature animals until one hundred and seventy days of age. They were then moved to the sow yards where they were given aural, visual and olfactory contact with sexually mature, intact boars. A boar was allowed into the gilt pen, under

TABLE 12

Daily rations for experimental pigs from 25 kg in weight until service (diet B only)

Weeks on trial	Ration kg/pig/day	Weeks on trial	Ration kg/pig/day
1	1.1	8	2.3
2	1.2	9	2.5
3	1.3	10	2.7
4	1.5	11	2.9
5	1.7	12	3.0
6	1.9		
7	2.1	Remains on 3 kg/day from week 13 until service	

Feed allowances for sows

Pregnancy

Parity	kg/diet
1	1.8
2	2.0
3	2.1
4	2.2
5	2.4
6	2.5

Lactation

Increasing by 400 g increments from 2 kg on Day 1 of lactation to 2 kg + 400 g/piglet suckled.

supervision, for a minimum of twenty minutes each day. A change in environment and close proximity to mature boars has been shown to stimulate the onset of puberty in the previously isolated gilt (Brooks and Cole, 1970).

The gilts were not mated at puberty, but at the second heat period. Two matings were given at an interval of twelve to twenty-four hours whenever possible. A service crate was available for use if any gilt was not able to accept the boar's full weight without aid. During pregnancy the animals were group-housed in sow yards with solid concrete floors, a strawed sleeping area and individual feeding stalls.

Seven days prior to parturition, females were moved to crates within the farrowing house, where they farrowed. They were moved to follow-on accommodation seven days post-partum. Weaning took place on one day a week (Thursday) when the piglets were thirty-five to forty-one days old. Sows were returned to the sow yards following weaning and given aural, visual and olfactory contact with sexually mature intact boars. Sows were served at the first post-weaning oestrus, two matings being given at an interval of twelve to twenty-four hours. Those sows failing to show oestrus by twenty-nine days post-weaning were treated with PG 600 (Intervet Labs Ltd.) to induce oestrus. In the event of failure to show oestrus in a further seven days the sows were slaughtered.

Routine Medication

All gilts were vaccinated twice against erysipelas prior to entering the sow yard then regularly vaccinated at each weaning.

The sows were routinely dosed with an oral broad spectrum anthelmintic prior to farrowing and were routinely treated against external parasites with Lindane (Cooper Lice and Mange Liquid).

Piglet Management

At birth the piglets were weighed, individually tattooed and their teeth clipped.

The piglets were given an iron dextran injection by seven days of age. A proprietary "creep" feed was freely available to the piglets from seven days of age.

The male piglets were castrated at three weeks of age. The piglets were weaned between thirty-five and forty-one days of age on a Thursday.

Boars

Three Large White boars and three Landrace boars were used. These were fed biotin-supplemented Diet B to prevent the development of biotin deficiency and therefore any confounding effect on the performance of the sows. The boars were routinely treated against external parasites.

Hoof Records

The feet of the sows were individually examined at one hundred and seventy days of age (when entering the sow yard) and at successive weanings. Detailed records were made of location and type of lesion present in the hoof.

The sows were nose-tethered and the feet lifted one by one, washed and dried. The injuries present in the horn side-wall and on the volar surface of each claw were diagramatically recorded on a record card (appendix 2). If necessary, comments were also made on the hooves, skin and coat condition. The injuries were categorised into two groups:

    i. Defects

        These were superficial injuries, either bruises, abrasions or cuts, the last category occurring only on the soft heel. These were not considered to be lesions in themselves, but were considered to be predisposing factors in lesion formation.

    ii. Lesions

        These were subdivided into erosions, cracks and overgrowths (Figure 3).

The hoof was divided into the following regions. The side-wall was composed of hard horn. The volar surface was divided into the heel and toe regions. The white line delineated the inward extent of the outer side-wall horn and the median toe horn. The heel, which was soft horn, merged posteriorly into the skin of the hock and anteriorly into the

FIGURE 3

Nomenclature used to describe foot lesions
(Modified after Brooks et al., 1977)
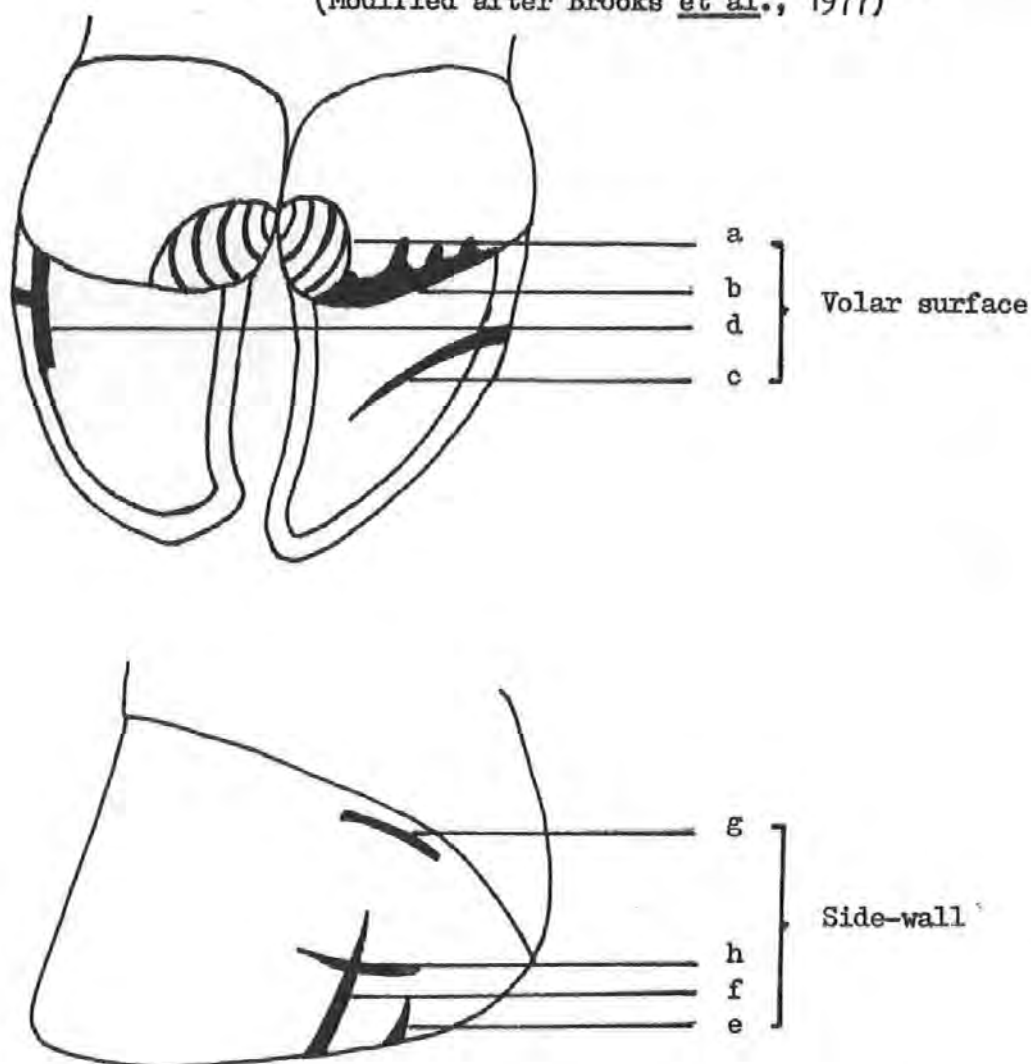
Erosion. This is predominantly on the soft heel often associated with the heel/toe junction region (a). It involves a loss of heel tissue and can result in secondary infection.

Crack. This is a splitting of the horn, hairline in the young animal, often resulting in extensive fissuring in the older animal with secondary infection. Found in all regions of the claw, mainly associated with erosions on the heel and heel/toe junction (b), also commonly found at the white line region on the axial and abaxial perimeter of the claw respectively (d). Abaxial white line lesions were frequently associated with cracking and underrunning of the side-wall (e and f). Median toe crack (c) is within the area delimited by the toe white line, mainly as a complication of the crack originating at the white line. Sidewall cracks are divided into vertical cracks (e and f) usually associated with cracks from the base of the feet. Horizontal cracks (g and h) are parallel to and having origin at the coronal band (g), and are often associated with vertical cracks (h).

harder toe horn at the heel/toe junction.

Feed and Blood Plasma Analyses

Blood plasma samples were taken from a representative sample of sows at each parity within two days of parturition and at weaning. A minor incision was made on the ventral side of the tail into the caudal vein and a ten ml sample of blood was removed, heparinised and centrifuged for fifteen minutes at 3000 rpm. The plasma was pipetted off, deep frozen and subsequently assayed for biotin using the method described by Frigg and Brubacker (1976). Samples of raw materials and complete diets were also analysed by the same method.

Measurements

Comprehensive records of sow reproductive performance were maintained. For gilts, the age at introduction to the boar and the interval from introduction to the boar to puberty were recorded. As gilts were not mated until the second oestrus period the intervals from introduction to the boar until second oestrus, first mating and first effective mating (ie mating resulting in parturition) were also recorded. In successive parities the interval from weaning to oestrus and to effective mating were also recorded.

The number and weight of piglets at birth, three weeks of age and at weaning were recorded.

Sows were weighed at the start of the trial, at the introduction to the boar, at puberty and at mating. In successive parities pre- and post- farrowing weights and weaning weights were also recorded.

The feet of sows were individually examined at introduction to the boar and at successive weanings and detailed records made of locations and type of lesion present on the hoof.

Details of treatments for anoestrus and sow removals were maintained throughout the trial.

Analysis of Data

The reproductive data were analysed in two ways. Within parities the

data were analysed using a one-way analysis of variance (Snedecor and Cochrane, 1956). For pooled data over the four parities, data were analysed using the least squares fitting constants procedure of Kempthorne (1952). This technique permitted the analysis of data having unequal treatment by parity subclasses and provided adjusted treatment means. Hoof data were analysed by chi-square and one-way analysis of varience (Snedecor and Cochrane, 1956).

RESULTS

Animals Providing Data

Of the eighty gilts initially allocated to the trial, 25 control and 22 supplemented sows completed four parities. The reasons for the removal from the trial of 15 control and 18 supplemented sows are summarised in Table 13. Reproductive failure was the primary cause for the removal of 7 control sows and 2 supplemented sows. Foot damage was not a primary cause of removal for any sow on either treatment. 12 supplemented and 4 control sows were removed due to locomotor problems. These sows suffered from abscesses of the shoulder, hip or spine which produced progressive recumbency. The abscesses resulted from physical damage to the hip and shoulder joints predominantly caused by sows falling when fighting following weaning or during mating. It was the opinion of the attendant veterinary surgeon that damage of this type was random and not in any way treatment related.

Biotin Level in Feed and Blood Plasma

The mean total biotin content of the vitamin premixes, raw materials and complete diets is summarised in Table 14. Some feed samples could not be assayed as they suffered from insect infestation. Of those raw materials assayed, the variation between samples was considerable. In the case of barley and white fish meal, the best samples contained 300% more biotin than the poorest samples. Unexpectedly the vitamin premix without biotin added gave positive values at low levels with much variation. A mean value of 308 mg/kg was achieved from the vitamin premix with biotin added.

46

TABLE 13

Summary of reasons for sow removal from trial

Reason for removal	Control	+ Biotin
Reproductive causes		
i. Anoestrus		
After 170 days	0	0
After first weaning	3	1
After second weaning	1	1
After third weaning	0	0
ii. Regular heat, no standing reaction	1	0
iii. Service, but repeated returning	2	0
Associated lameness & reproductive causes		
i. Repeated returning & lameness in joints	1	0
ii. Abortion & lameness in joints	1	3
Lameness		
i. Feet alone	0	0
ii. Causes, other than in feet	3	9
Other causes	3	4
Total	15	18

Summary of sows completing parities

Parity	Control	+ Biotin
First	36	32
Second	30	29
Third	26	23
Fourth	23	21

TABLE 14

Total biotin levels in feed

Description of feed	Calculated level[1] (μg/kg)	Mean assay result (μg/kg)	Range (μg/kg)
Wheat	100	90	76-113
Barley	140	129	89-236
White fish meal	200	126	70-227
Vitamin premix – biotin	0	238	28-1155
Vitamin premix + biotin	350[2]	308[2]	281-337[2]
Diet A (no added biotin)	122	116	88-153
Diet A (plus biotin)	472	430	356-484
Diet B (no added biotin)	120	104	88-172
Diet B (plus biotin)	558	427	372-486

[1] Levels are calculated from published values.

[2] Figures quoted in mg/kg.

Not all blood samples recorded values as the sampling technique failed to provide sufficient blood.for assay on some occasions and haemolysis of red blood cells occurred in other samples. The samples assayed showed that plasma biotin levels in sows were influenced by dietary treatment (Table 15). The mean plasma biotin level in supplemented sows was significantly higher (p<0.01) than that of the control sows at both pre-farrowing and weaning. Both treatments exhibited a large range in values at both times. The plasma biotin levels between pre-farrowing and weaning were not significantly different within treatments, although the control sows gave some evidence of a slight reduction.

Service Interval Data

1. Interval from introduction to boar to first mating

All gilts entered the sow yard and were introduced to the boar at 170 days of age to induce puberty. The mean days to puberty, following this age, were not significantly influenced by treatment (Table 16). 71% of control and 75% of supplemented animals attained puberty within 10 days (Figure 4). Similarly at day 30, 54% of control gilts and 60% of supplemented gilts had conceived (Figure 5). Despite this, mean days to effective service were not significantly influenced by treatment.

2. Interval from weaning to remating

There was a notable tendency for biotin-supplemented sows to have shorter intervals between weaning and oestrus and between weaning and effective mating than control sows (Table 17). This difference was particularly marked after the sows were weaned from their first and second litters.

Pooled data for the first three weanings indicated that supplemented sows returned to oestrus 2.9 days sooner than controls and conceived 6.1 days sooner (p<0.05). Not only did the supplemented sows have shorter mean intervals to oestrus and mating but also a higher percentage of returns to oestrus and conception occurred within 10 days of weaning (Table 18 and Figures 6 to 11 incl.). Only following the third weaning did the control sows have a higher percentage of returns

49

TABLE 15

Biotin levels in blood plasma (ng/100 ml)

Time of sampling		Treatment			Level of significance between treatments
		Control	+ Biotin	SED	
Pre-farrowing					
i.	Mean	111	192	24.8	* *
ii.	Range	43-264	65-303		
iii.	Sample (No)	15	13		
Weaning					
i.	Mean	103	196	31.3	* *
ii.	Range	39-307	92-546		
iii.	Sample (No)	19	15		

TABLE 16

Summary of interval to puberty and first service data for all sows

Event	Control	+ Biotin
		(Days)
Age at starting trial		81.6 (80)
Age at introduction to boar	170 (38)	170 (37)
Period from introduction to boar to:		
i. Puberty	11.8 ± 1.9 (38)	11.0 ± 2.1 (37)
ii. Second oestrus	34.1 ± 2.0 (38)	33.3 ± 2.1 (36)
iii. Service	35.4 ± 2.1 (37)	35.8 ± 2.6 (35)
iv. Effective service	36.5 ± 2.1 (36)	35.8 ± 2.6 (35)

Note: Figures in parenthesis indicate sample size.

TABLE 17

Summary of weaning to service intervals for all parities

Event	Control	+ Biotin
Period from first weaning to:	(Days)	
i. Oestrus	16.2 ± 3.0 (33)	11.8 ± 1.9 (30)
ii. Service	16.2 ± 3.0 (33)	14.5 ± 2.9 (30)
iii. Effective service	21.9 ± 5.2 (31)	14.9 ± 3.0 (29)
Period from second weaning to:		
i. Oestrus	13.3 ± 2.8 (28)	8.7 ± 1.9 (24)
ii. Service	14.1 ± 2.8 (28)	8.7 ± 1.9 (24)
iii. Effective service	17.6 ± 4.4 (28)	9.9 ± 2.8 (22)
Period from third weaning to:		
i. Oestrus	6.8 ± 0.8 (27)	6.8 ± 1.3 (23)
ii. Service	6.8 ± 1.1 (27)	6.8 ± 1.3 (23)
iii. Effective service	10.0 ± 3.5 (27)	6.9 ± 1.4 (21)
Period from fourth weaning to:		
i. Oestrus	6.9 ± 1.3 (18)	6.2 ± 1.1 (20)
ii. Service	6.9 ± 1.3 (18)	6.2 ± 1.1 (20)
iii. Effective service	6.2 ± 0.7 (15)	5.5 ± 0.4 (16)

Event	Control	SED	+ Biotin
Overall period (first to third weaning):			
i. Oestrus	11.9 (88)	1.7	9.0 (77)
ii. Service	12.4 (88)	1.5	10.0 (77)
iii. Effective service	16.5 (86)	1.4	10.4 (72)

Note: Figures in parentheses indicate sample size.
Overall weaning to effective service period is significantly different ($p < 0.05$).
Period from fourth weaning is outside of the trial period.
Period to effective service includes only those matings resulting in parturition.

52

TABLE 18

Percentage of sows returning to oestrus and conceiving within 10 days of weaning

Event	% sows achieving oestrus		% sows successfully mated	
	Control	+ Biotin	Control	+ Biotin
First weaning	63	73	57	68
Second weaning	68	88	66	88
Third weaning	96	91	85	91

FIGURE 4

The daily cumulative increase in the percentage
of gilts attaining puberty after introduction
to boar at 170 days of age
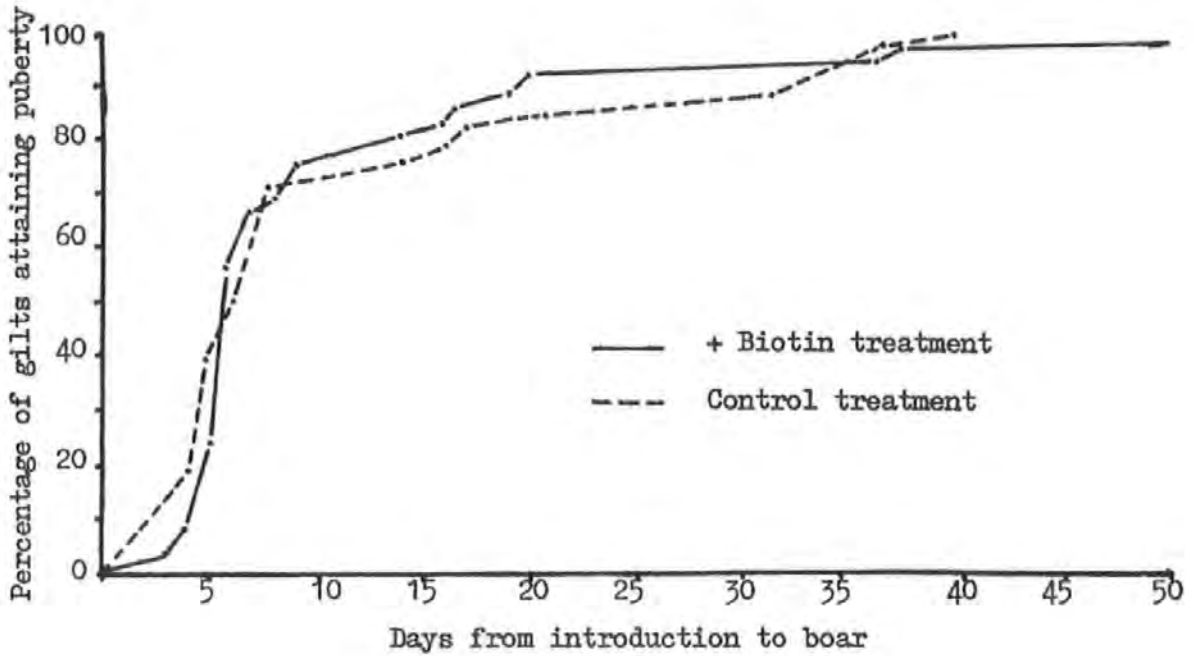
FIGURE 5

The daily cumulative increase in the percentage
of gilts effectively served after introduction to
boar at 170 days
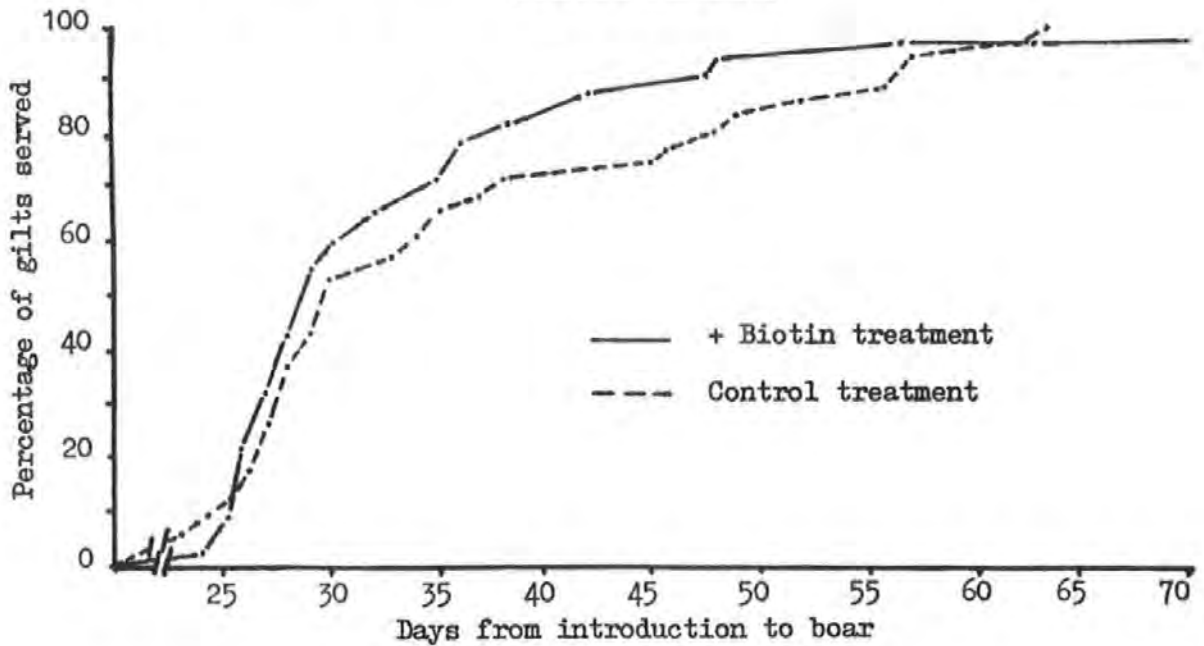
FIGURE 6

The daily cumulative increase in percentage
of gilts attaining oestrus at first weaning
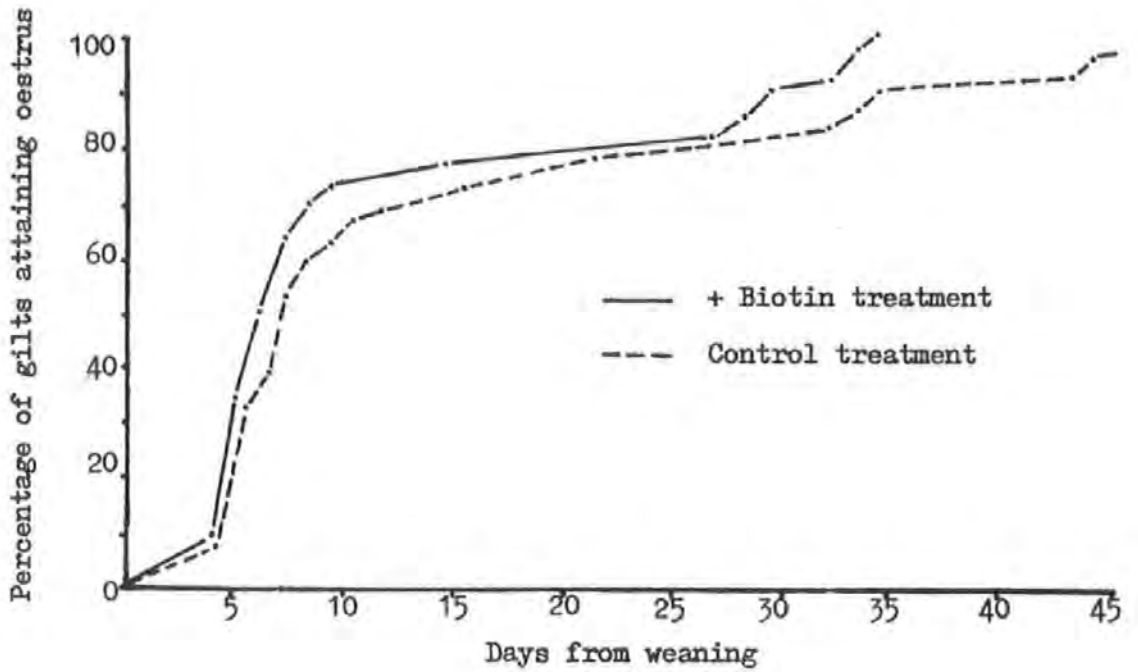
FIGURE 7

The daily cumulative increase in percentage
of sows attaining effective service at first weaning
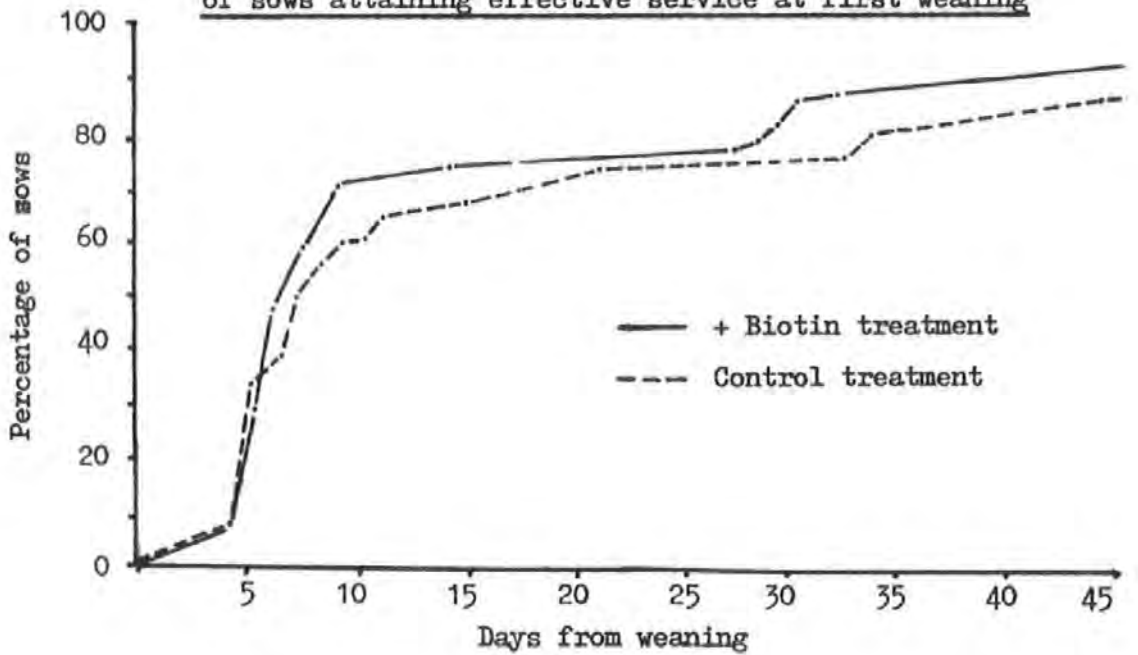
FIGURE 8

The daily cumulative increase in percentage of
sows attaining oestrus at second weaning
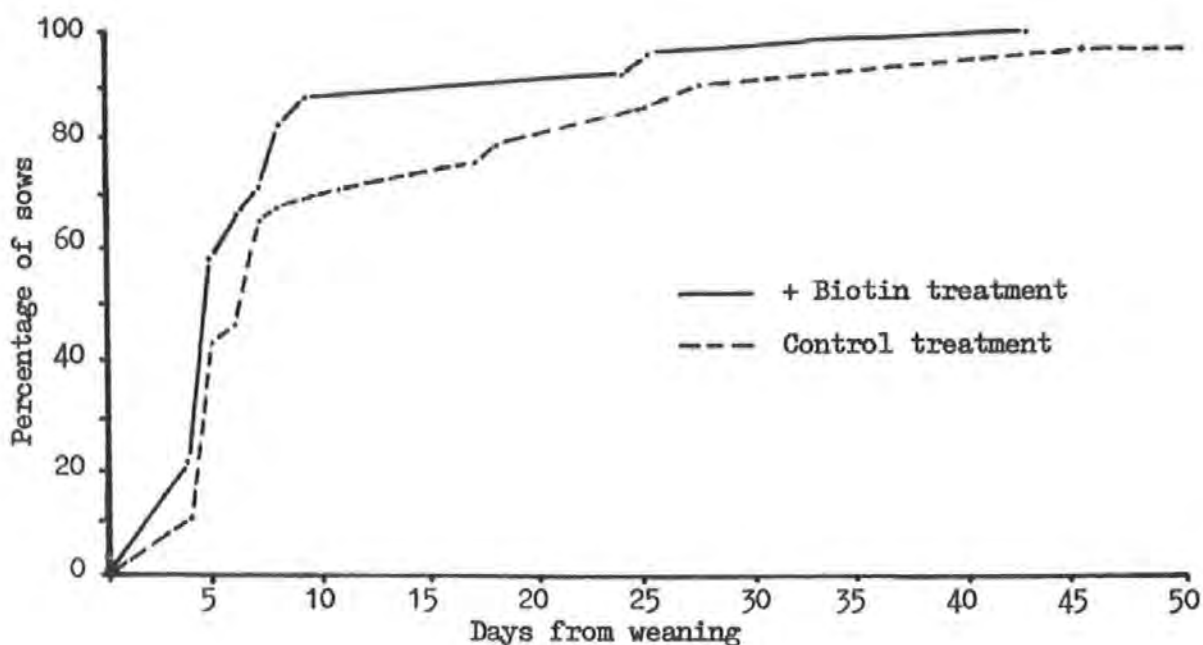
FIGURE 9

The daily cumulative increase in percentage of
sows attaining effective service
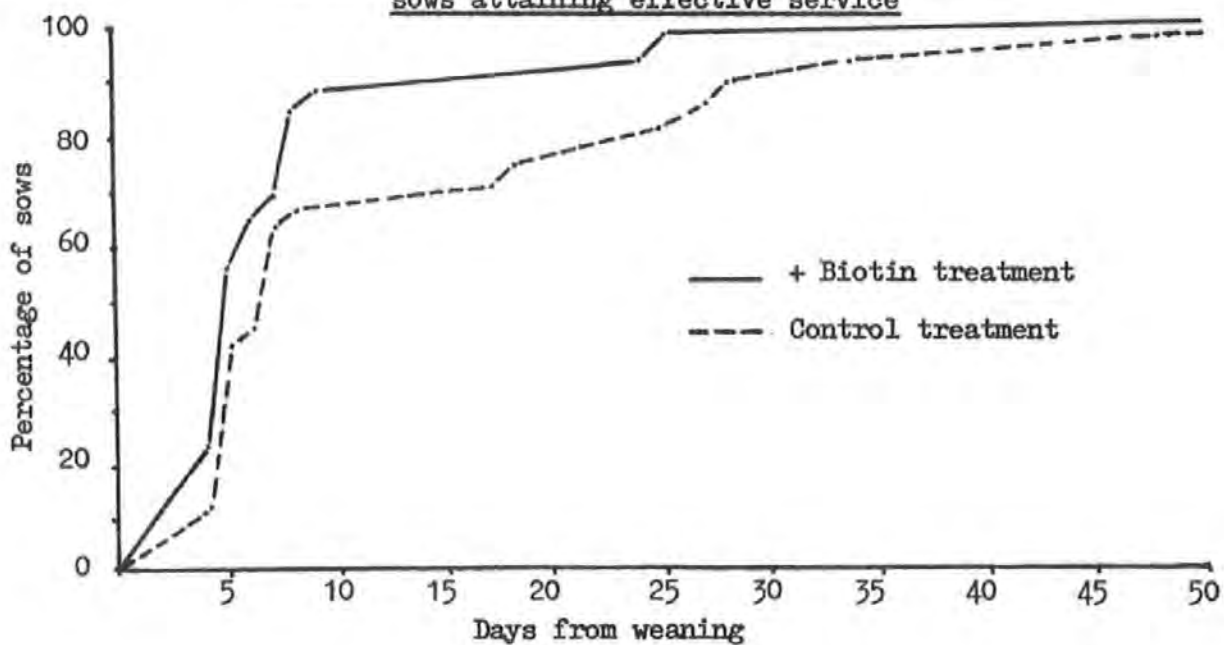
FIGURE 10

The daily cumulative increase in percentage of sows
attaining oestrus at third weaning
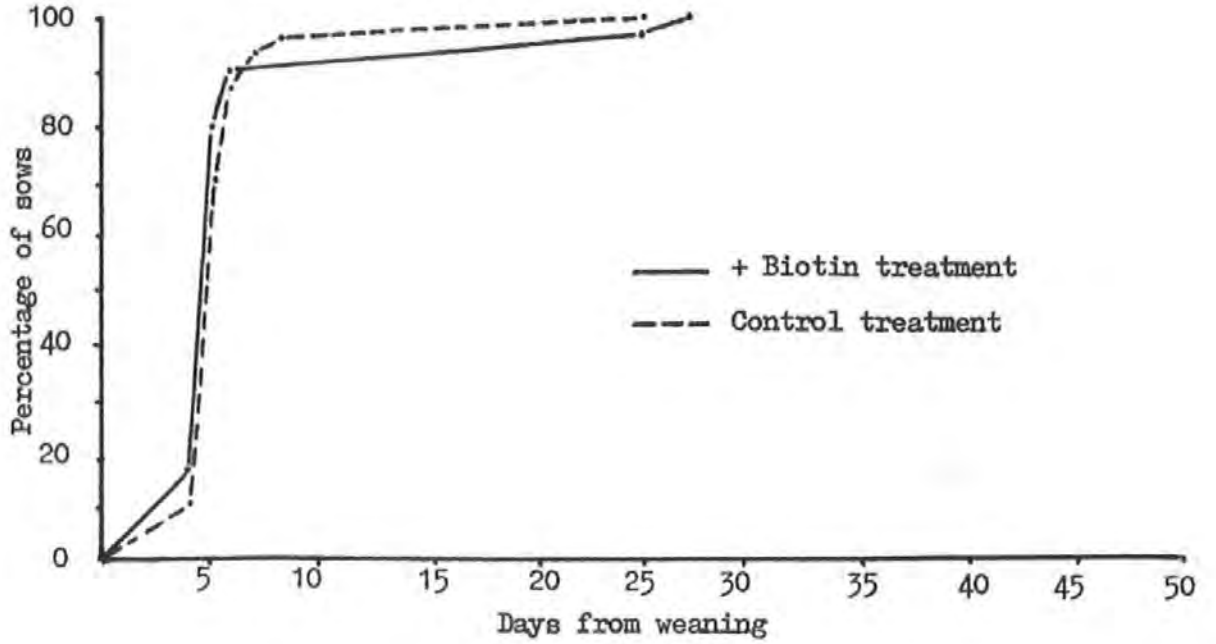
FIGURE 11

The daily cumulative increase in percentage of sows
attaining effective service

57
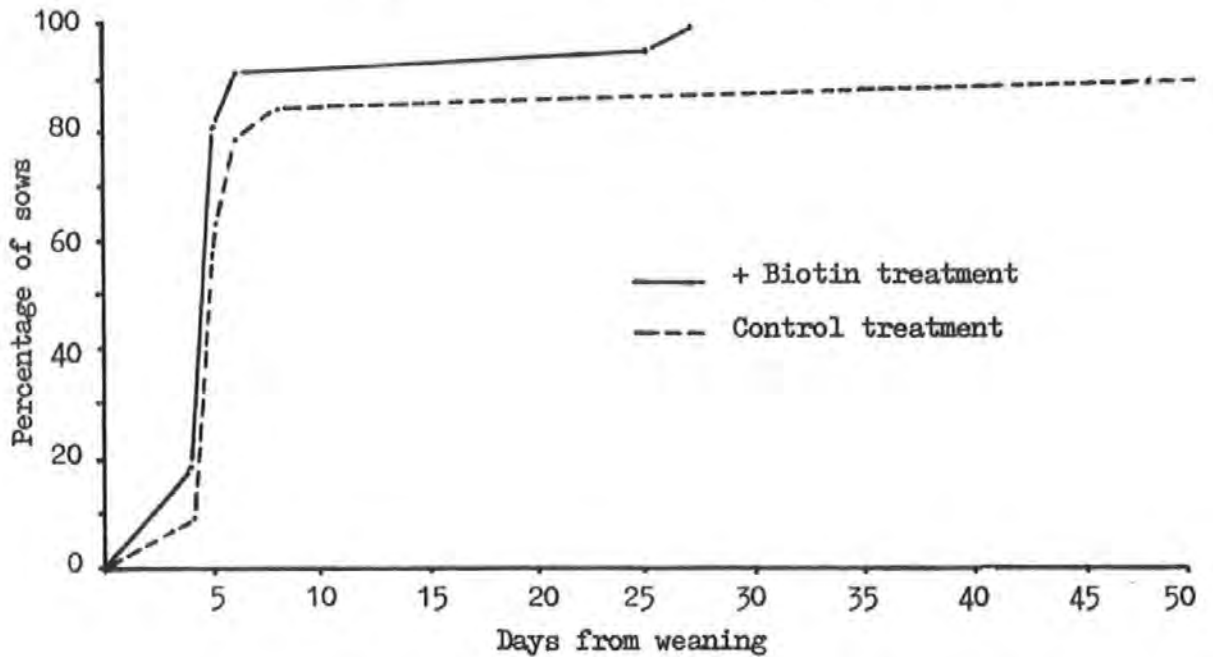
to oestrus within 10 days of weaning. However, despite this a higher percentage of supplemented animals conceived within 10 days of weaning (Figures 10 and 11).

In accordance with the management policy for the herd, sows which failed to return to oestrus within 29 days of weaning were injected with PG 600 (Intervet) to induce oestrus; 4 control and 2 supplemented sows failed to respond to treatment after 7 days and were culled. However, the remaining 11 control and 4 supplemented sows were successfully treated during the course of the experiment (Table 19).

Finally it should be noted that conception rate was high for all sows on the trial. Notwithstanding this fact there were treatment differences. Excluding those returns to service following treatment for oestrus induction, 6 control sows failed to hold to service in the course of the trial whereas none of the supplemented animals returned to service.

Litter Performance Data

1. Number born and reared

The number of piglets born/litter increased with age of the sow. The performance of the first parity supplemented sows was inferior to that of the control sows. This trend was reversed with the second parity (9.5 vs 10.6 piglets/litter for control and supplemented sows respectively) and thereafter supplemented sows produced more piglets in each subsequent parity (Table 20 and Figure 12). A proportion of all the piglets born were born dead, but the means for those born alive showed a similar trend to those for total births. The overall adjusted treatment means indicate an advantage of 0.2 live piglets/litter for the supplemented sows.

The number of piglets weaned also showed an overall advantage of 0.3 piglets/litter in favour of the supplemented sows. The supplemented sows though slightly inferior at the first parity were markedly superior at the second and fourth parities (8.7 vs 9.4 and 8.6 vs 9.5 piglet/litter for control and supplemented groups respectively). The third parity data were similar for the two treatments.

58

TABLE 19

Oestrus induction and returns in experimental sows

Treatment group	Period	Oestrus induction			Other returns to first service
		Service no return	Return to first service following treatment	Ineffective – remained anoestrus[1]	
Control	After puberty	–	–	–	2 (36)
	After first weaning	5	3	3	1 (33)
	After second weaning	2	1	1	1 (28)
	After third weaning	0	0	0	2 (27)
+ Biotin	After puberty	–	–	–	0 (35)
	After first weaning	3	0	1	0 (30)
	After second weaning	0	1	1	0 (29)
	After third weaning	0	0	0	0 (23)

Note: Figures in parentheses indicate sample size.

[1] All sows not responding to treatment were culled.

59

TABLE 20

Effect of dietary biotin supplementation and age of sow on litter performance

| Treatment | Treatment means (overall, parity 1 to 4) | | | | | | | | | | Between treatment means SED | | | | |
| | Overall | | 1 | | 2 | | 3 | | 4 | | | | | | |
	C	S	C	S	C	S	C	S	C	S	Overall	1	2	3	4
Number of piglets born	10.8	10.9	10.0	9.3	9.5	10.6	11.8	12.0	11.7	12.0	0.4	0.7	0.6	0.9	1.0
Number of piglets born alive	10.2	10.4	9.5	8.9	9.3	10.2	11.4	11.4	10.6	11.3	0.4	0.6	0.6	0.8	0.9
Number of piglets at 3 weeks	8.7	9.0	8.2	7.9	8.7	9.4	9.6	9.5	8.6	9.5	0.3	0.6	0.6	0.8	0.8
Number of piglets at 5 weeks	8.7	9.0	8.1	7.8	8.7	9.4	9.5	9.5	8.6	9.4	0.3	0.6	0.6	0.8	0.8
Mortality as percentage live births	-	-	14.7	12.4	6.5	7.8	16.7	16.7	18.9	16.8	-	-	-	-	-
Litter birth wt (kg)	14.7	15.2	12.9	12.3	14.5	15.5	13.7	16.1	15.5	17.1	0.5	0.8	0.8	0.9	1.1
Litter 3 week wt (kg)	45.0	46.1	39.9	38.2	44.9	48.8	49.8	48.9	44.9	48.9	1.5	2.8	2.9	2.9	3.3
Litter 5 week wt (kg)	73.6	76.2	62.0	62.5	75.3	80.1	81.0	79.1	75.7	83.6	2.1	4.8	4.9	6.5	6.5
Piglet birth wt (kg)	1.42	1.44	1.34	1.33	1.50	1.49	1.40	1.44	1.44	1.51	0.02	0.06	0.07	0.06	0.08
Piglet 3 week wt (kg)	5.2	5.2	4.9	4.9	5.3	5.2	5.2	5.3	5.3	5.3	0.0	0.0	0.1	0.1	0.0
Piglet 5 week wt (kg)	8.6	8.6	7.8	8.2	9.0	8.7	8.6	8.5	9.0	9.2	0.2	0.4	0.4	0.5	0.5

60

FIGURE 12

Effect of treatment and parity of sow on the distribution of litter size

i. At birth
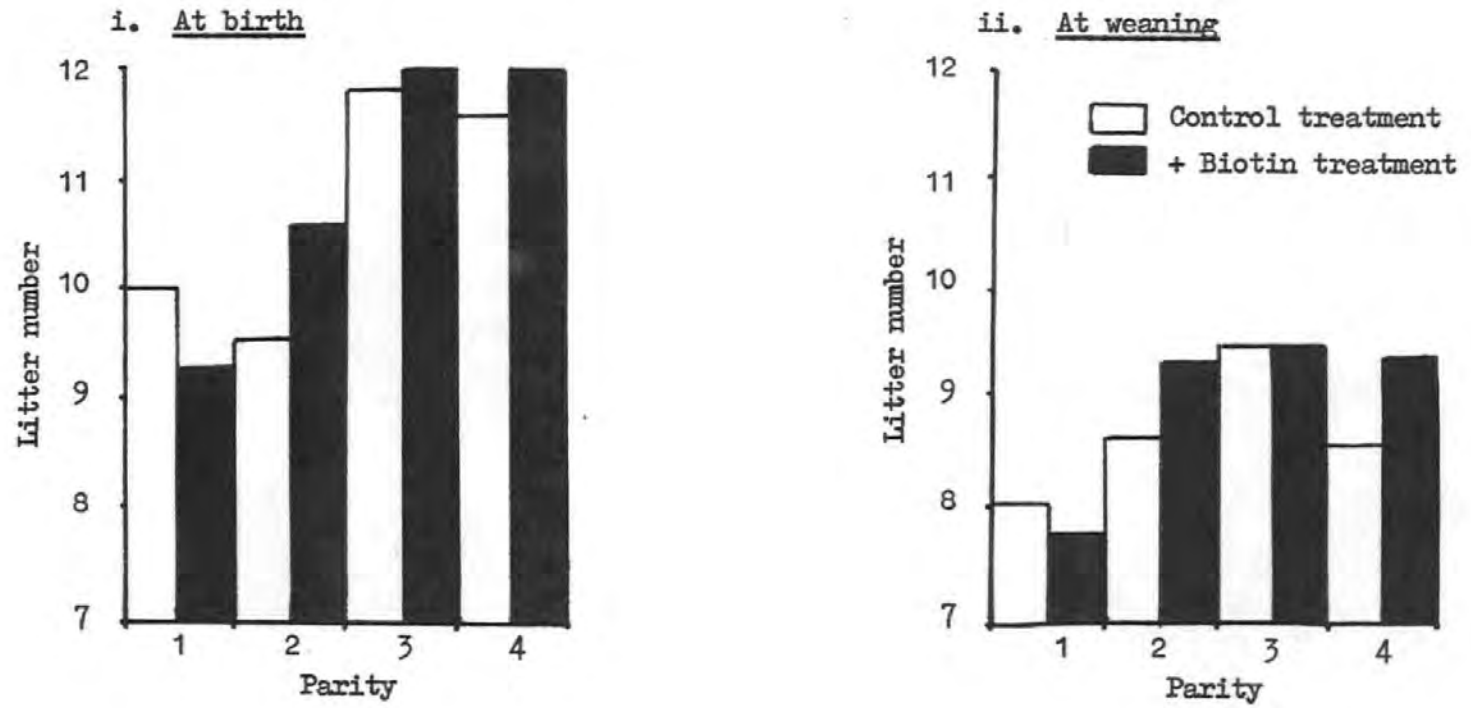
ii. At weaning

The number of piglets/litter at 3 weeks was the same as at weaning. In fact most mortality occurred within the first few days of a piglet's life. The level of mortality was high, partly due to the occasional outbreak of MMA that afflicted both treatments similarly. Overall, mortality tended to be higher in the control group, even if two third parity control litters which died within a week of birth, were excluded from the results. If included, the percentage mortality would have been 22.8% compared with 16.7% for the supplemented group.

2. Piglet and litter weights

The supplemented sows produced a greater total weight of piglets. Overall at birth they produced 0.5 kg/litter more (Table 20); this difference was maintained at weaning giving an advantage of 2.6 kg/litter to the supplemented sows.

The control litters were heavier at birth in the first parity (not at weaning) but were lighter from the second parity (Figure 11). Although this difference was not statistically significant, the cumulative change in litter weight with parity shows that the rate of increase in the supplemented sows was higher than for the control sows for both weight at birth and weight at weaning (Figure 12).

The individual piglet weights per parity at birth, three weeks and at weaning were similar for both treatments.

3. Results for sows completing the four parities

A comparison of litter productivity was made between sows from both treatments which completed four parities (Table 21). Little difference was exhibited between treatments for total number and weight of pigs born. However, the supplemented sows weaned 2.6 ± 1.83 more pigs than control animals and the total weight of pigs weaned was 27.1 ± 12.7 kg greater ($p < 0.05$).

Litter Production in Unit Time

Herd production figures (as rate of piglet production per litter per parity per year) can be calculated from the previous litter performance and service interval data for each treatment (Table 22 and

FIGURE 13

Effect of treatment and parity of sow on the distribution of litter weight

i. At birth

ii. At weaning

Control treatment

+ Biotin treatment
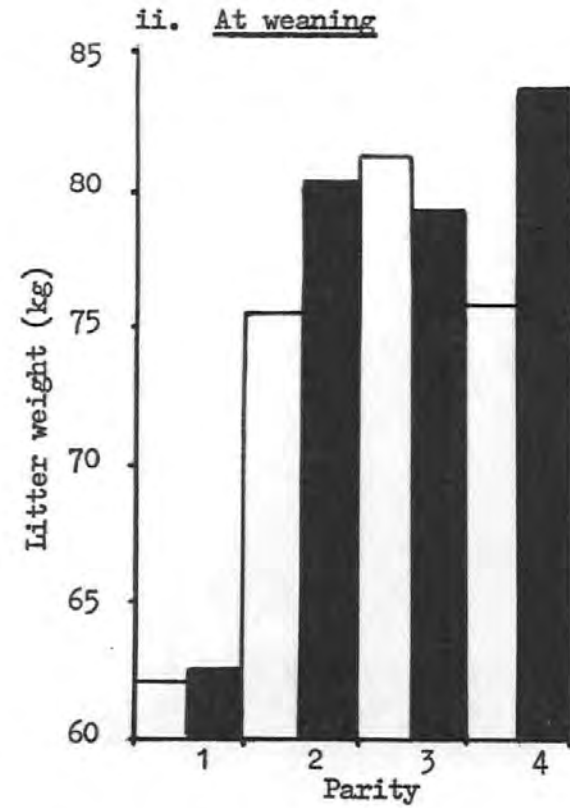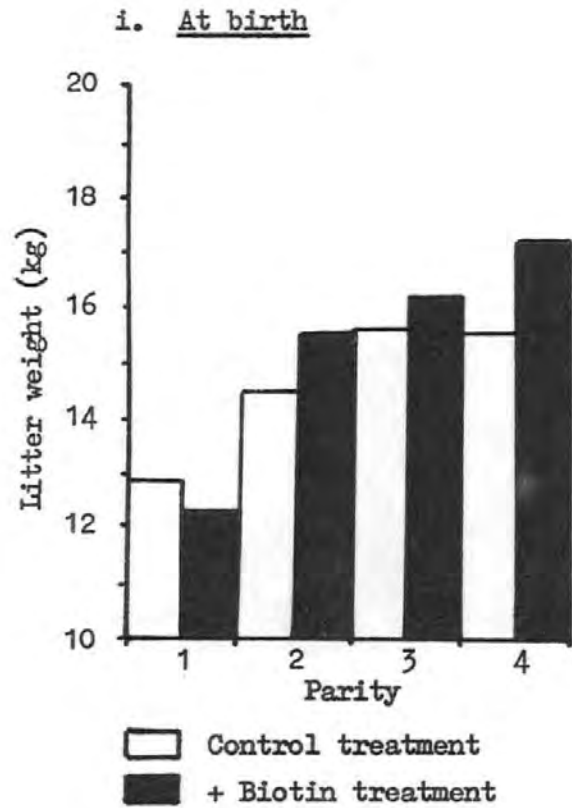
FIGURE 14

Effect of treatment and parity of sow on the cumulative average litter weight

i. At birth
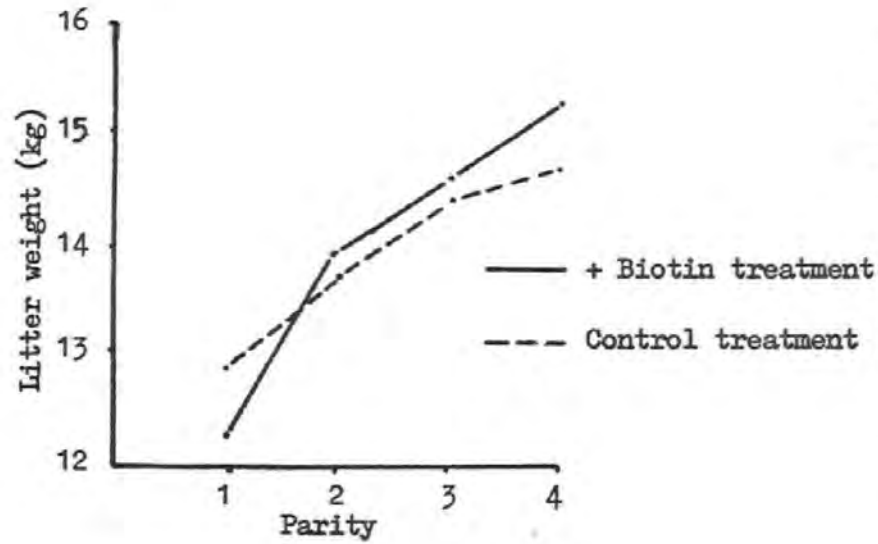
ii. At weaning
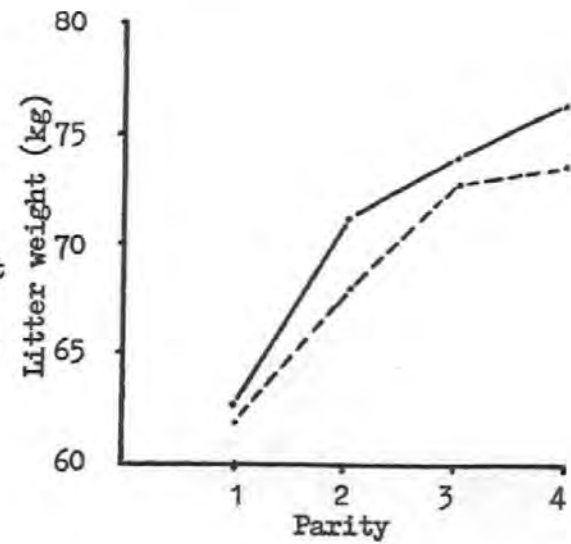
TABLE 21

Effect of treatment on litter productivity of sows completing four reproductive cycles

(Cumulative production over first four parities)

	Control	+ Biotin	SED
Number of animals	23	21	
Total pigs born	43.3	44.4	2.48
Total pigs weaned	34.5	37.1	1.83
Total weight of pigs born (kg)	59.1	62.5	2.60
Total weight of pigs weaned (kg)	288.2	315.3	12.66[1]

[1]Difference significant at $p < 0.05$.

TABLE 22

Piglet production rate per sow per parity per year

	Control	+ Biotin
	(Piglets/sow/year)	
First parity		
i. Piglets born	19.4 ± 0.9 (36)	17.9 ± 1.0 (32)
ii. Piglets born alive	18.5 ± 0.8 (36)	17.2 ± 0.9 (32)
iii. Piglets weaned	15.8 ± 0.8 (36)	15.0 ± 0.9 (32)
Second parity		
i. Piglets born	20.8 ± 1.2 (30)	23.3 ± 0.9 (29)
ii. Piglets born alive	20.3 ± 1.1 (30)	22.7 ± 0.9 (29)
iii. Piglets weaned	18.9 ± 1.1 (30)	20.9 ± 0.9 (29)
Third parity		
i. Piglets born	26.0 ± 1.4 (28)	27.6 ± 1.4 (23)
ii. Piglets born alive	24.9 ± 1.4 (28)	26.1 ± 1.3 (23)
iii. Piglets weaned	19.6 ± 1.4 (28)	21.7 ± 1.5 (23)
Fourth parity		
i. Piglets born	26.6 ± 1.7 (23)	27.5 ± 1.5 (21)
ii. Piglets born alive	23.8 ± 1.4 (23)	26.3 ± 1.5 (21)
iii. Piglets weaned	19.3 ± 1.1 (23)	21.9 ± 1.4 (21)
All four parities		
i. Piglets born	22.7 ± 0.7 (117)	23.8 ± 0.7 (105)
ii. Piglets weaned	18.2 ± 0.6 (117)	19.5 ± 0.6 (105)

Note: Figures in parentheses indicate sample size.

FIGURE 15

Effect of treatment and parity of sow on productivity in terms of piglets per sow per year
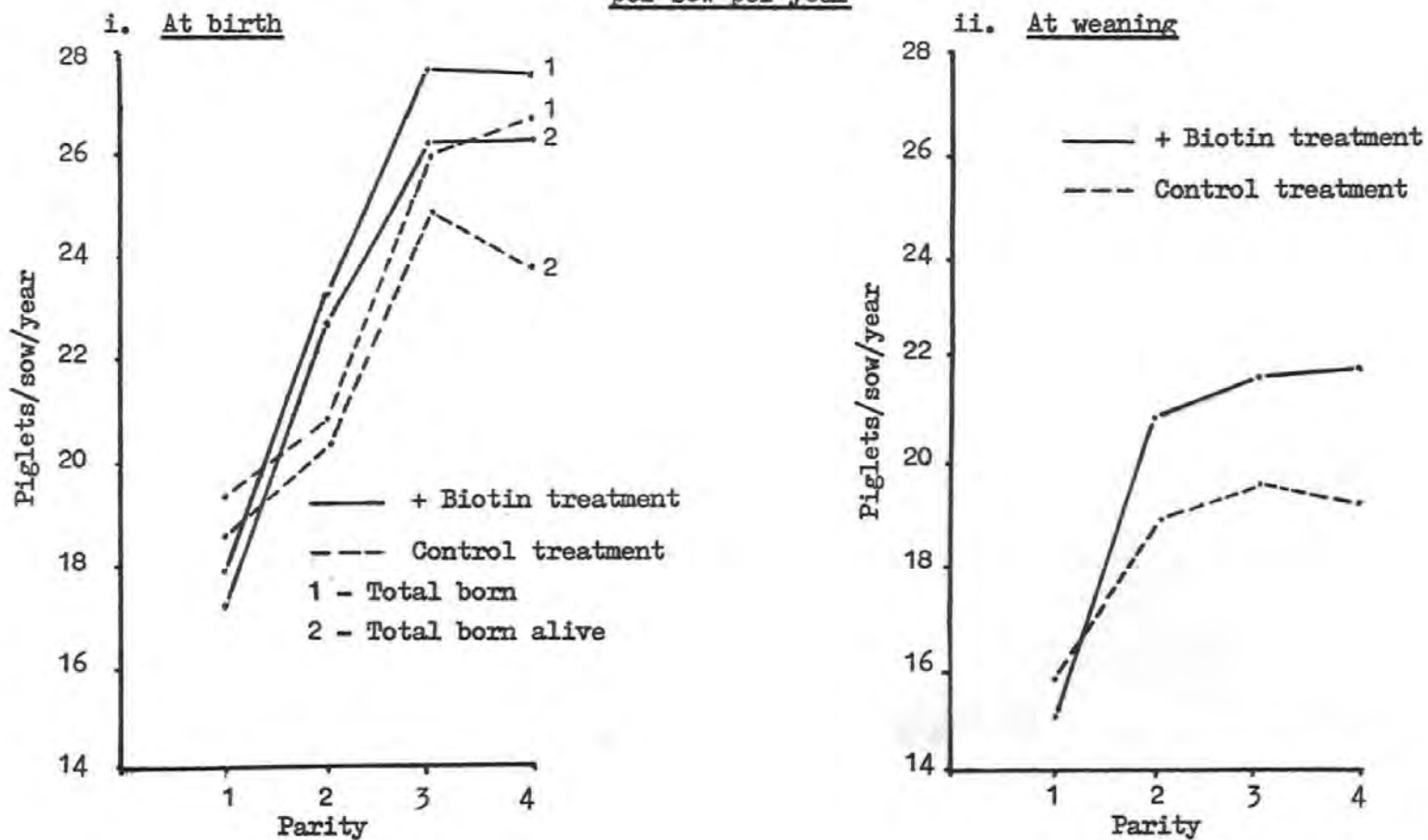
Figure 15).

The control sows had a higher rate of production for the first parity
at birth and at weaning. The supplemented sows had a higher production
rate for the other parities, so that the difference in performance in
favour of the supplemented treatment at weaning increased with each
additional parity. This is clearly illustrated in Figure 16 which
shows the cumulative change in production per parity. At the fourth
parity the cumulative production was 18.7 vs 20.5 piglets/sow/year for
control and supplemented pigs respectively. This would be equivalent
to an extra 1.8 piglets/sow/year produced by the supplemented sows in a
herd with the age profile produced in this trial.

Using the data from those sows which completed four reproductive cycles
it was possible to calculate daily productivity for both treatments
from first mating to weaning of the fourth litter (Table 23). More
supplemented piglets were born and weaned per day. The weight of pig-
lets born per day was slightly greater and the weight of piglets weaned
per day was significantly ($p < 0.05$) greater in the supplemented
treatment. In terms of annual productivity, biotin supplementation
resulted in the production of 1.42 ± 1.02 more pigs weaned per sow per
year and 17.3 ± 7.4 kg more weight of weaned pig per sow per year
($p < 0.05$).

The major production losses were due to loss of live piglets to weaning
(Table 24). Production losses due to the time the sows spent in the
herd after weaning and prior to culling accounted for a small proportion
of the total losses. It was estimated that a loss in piglets/sow/year
which could be attributed to reproductive causes, such as anoestrus, was
0.6 vs 0.1 for the control and supplemented sows respectively. This can
be compared with the losses through all causes of 15.2 vs 13.9 piglets/
sow/year for the control and supplemented sows respectively.

Sow Weight Change

The analysis of the weight data for all sows showed no statistically
significant difference at any of the times readings were taken (Table 25).
The first parity readings were similar for both groups, the control sows
were slightly heavier for the second and third parities. The rate of
growth was greatest in the gilt and reduced with age. Little growth was

FIGURE 16

Effect of treatment and parity of sow on the cumulative change in
productivity in terms of piglets per sow per year

i. At birth

ii. At weaning
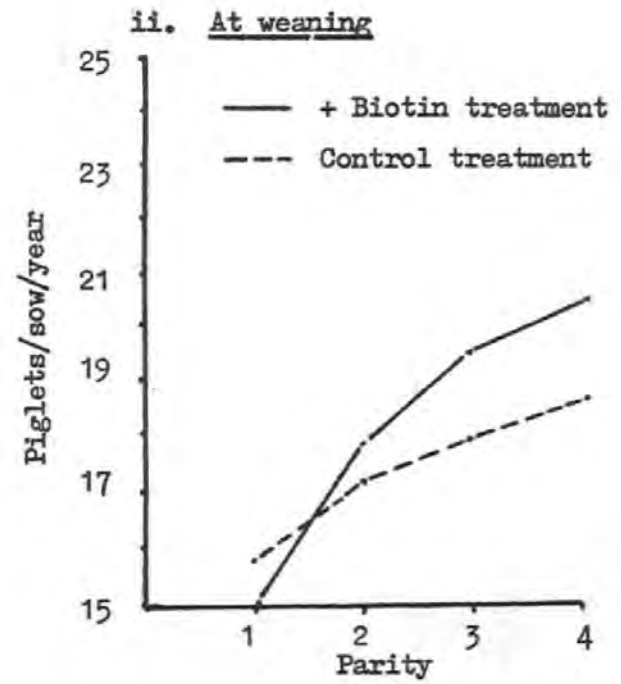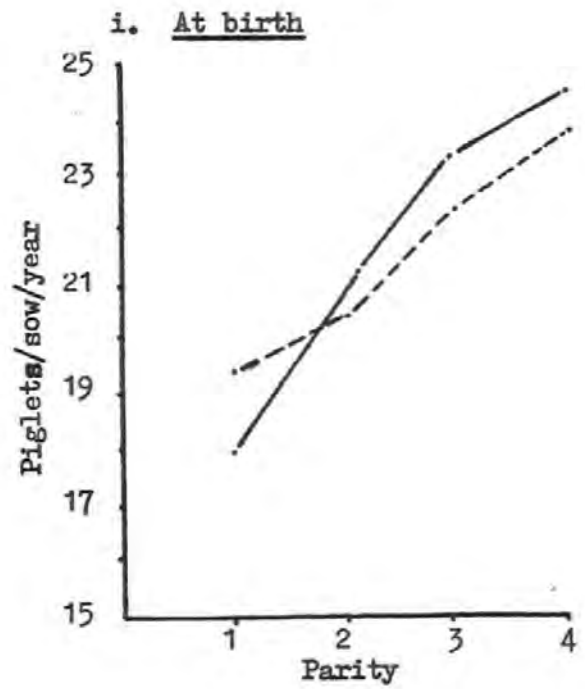
TABLE 23

Effect of biotin supplementation on productivity per day and per sow per year[1]

	Control	+ Biotin	SED
Number of pigs born/d	0.0632	0.0659	± 0.0040
Number of pigs weaned/d	0.0509	0.0548	± 0.0028
Weight of pigs born (g/d)	86.4	93.5	± 4.6
Weight of pigs weaned (g/d)	420.9	468.4	± 20.3[2]
Pigs born/sow/year	23.08	24.05	± 1.45
Pigs weaned/sow/year	18.58	20.00	± 1.02
Weight of pigs born/sow/year (kg)	31.52	34.14	± 1.70
Weight of pigs weaned/sow/year (kg)	153.64	170.97	± 7.42[2]

[1]Calculated from herd entry at 170 d of age to weaning of fourth litter for sows completing four parities only.

[2]Difference significant at $p < 0.05$.

TABLE 24

Piglet production losses

Loss factor	Control	+ Biotin
	(Pig/sow/year)	
Live piglets lost to weaning		
i. First parity	2.7	2.2
ii. Second parity	1.4	1.8
iii. Third parity	5.3	4.4
iv. Fourth parity	4.5	4.4
Culling (weaning to culling period)		
i. Reproductive	0.6	0.1
ii. Other	0.7	1.0
Total	15.2	13.9

TABLE 25

Summary of weight data for all sows

Event	Control (kg)	+ Biotin (kg)
Starting trial	26.0	
Entry to sow yard	81.8 ± 1.5	81.3 ± 1.2
Puberty	88.7 ± 1.5	89.6 ± 1.8
First service	106.4 ± 1.5	106.6 ± 1.6
First parity		
i. Pre-farrowing	154.4 ± 1.9	155.4 ± 2.2
ii. Post-farrowing	141.2 ± 5.0	147.4 ± 1.9
iii. Weaning	141.4 ± 1.9	141.7 ± 1.7
Second parity		
i. Pre-farrowing	184.2 ± 2.7	182.1 ± 2.8
ii. Post-farrowing	173.5 ± 3.0	175.8 ± 3.2
iii. Weaning	168.3 ± 2.5	162.2 ± 2.4
Third parity		
i. Pre-farrowing	200.1 ± 4.3	197.1 ± 3.4
ii. Post-farrowing	195.3 ± 7.9	187.3 ± 3.8
iii. Weaning	174.3 ± 3.7	169.7 ± 3.5
Fourth parity		
i. Pre-farrowing	200.4 ± 4.9	202.7 ± 3.4
ii. Weaning	167.3 ± 12.6	180.9 ± 5.9

exhibited between the third and fourth parities.

Hoof studies

1.   Observations at 170 days of age

The condition of the hooves was first assessed when the animals were
170 days of age. Few claws were observed to have defects in the control
(c) and supplemented (s) treatments (c = 1.8 $\pm$ 0.3; s = 1.5 $\pm$ 0.2;
Table 26) and at least 50% of both treatments had either no or one claw
with defects (Figure 17). Similarly the mean number of defects per gilt
was only slightly greater in the control group than the supplemented
group (c = 1.87; s = 1.64; Table 26). Most defects occurred on the
volar surface, primarily on the soft heel and heel/toe junction, but
damage was also observed in the white line region of the toe (Table 27).

At 170 days of age the degree of damage from lesions was similar for
both treatments with 2.8 $\pm$ 0.3 claws per animal affected, at a rate of
5.80 and 5.49 lesions/sow for the control and supplemented treatments
respectively (Table 26). Compared with later assessments, a higher
proportion of gilts had no lesion on any claw (13.2% and 15.0% for
control and supplemented gilts respectively) and only control gilts had
seven or eight claws with lesions (c = 7.9%; Figure 20). The lesions
occurred predominantly in the toe and side-wall regions with the most
frequent lesions being vertical cracks in the side-wall and cracks in
the white line regions of the toe for both groups. Although these
lesions tended to be "hairline" only, significant differences were
observed between treatments. The white line of the toe had sig-
nificantly more lesions in the supplemented gilts (c = 2.47 vs s = 3.00;
p < 0.05) whereas the side-wall showed significantly more lesions in the
control group (c = 2.74 vs s = 2.08; p < 0.05). There was little
evidence of secondary infection at this stage.

The level of damage by lesions to the inner and outer claws was similar
for each claw between treatments, except for the hind inner claws
(Table 29). Here the supplemented treatment had significantly less
lesions/claw than the control treatment (c = 0.66; s = 0.45; p < 0.05).
Comparisons of the inner and outer claws of the fore and hind feet
respectively showed that the inner claws had significantly less lesions
within each treatment (p < 0.05 or greater).

73

TABLE 26

Effect of dietary biotin supplementation and age on the mean number of
defects and lesions affecting the claw of the sow

Period of examination	Treatment	Claws/sow with defects	Defects /sow [1]	Claws/sow with lesions	Lesions /sow [2]	Sows completing four parities		
						Claws/sow with defects	Claws/sow with lesions	Lesions /sow [2]
170 days	Control (38)	1.8 ± 0.3	1.87	2.8 ± 0.3	5.80	1.4 ± 0.3	3.0 ± 0.5	6.21^a
	+ Biotin (39)	1.5 ± 0.2	1.64	2.8 ± 0.3	5.49	1.7 ± 0.3	2.5 ± 0.5	4.90^a
First weaning	Control (36)	2.6 ± 0.3	2.97	5.4 ± 0.3^d	13.45^c	2.3 ± 0.4	5.3 ± 0.3^d	13.21^c
	+ Biotin (32)	2.6 ± 0.3	3.38	4.5 ± 0.3^d	9.79^c	2.8 ± 0.4	4.4 ± 0.4^d	8.34^c
Second weaning	Control (31)	2.4 ± 0.3	2.80	5.8 ± 0.3	15.01^b	2.6 ± 0.4	5.8 ± 0.4	15.21^a
	+ Biotin (29)	2.6 ± 0.3	2.96	5.4 ± 0.4	12.96^b	2.8 ± 0.4	5.3 ± 0.4	13.10^a
Third weaning	Control (27)	2.1 ± 0.2	2.35	6.4 ± 0.3	17.96^c	2.1 ± 0.2	6.4 ± 0.3	18.33^c
	+ Biotin (22)	1.8 ± 0.3	1.87	5.7 ± 0.4	14.41^c	1.7 ± 0.3	5.7 ± 0.4	14.19^c
Fourth weaning	Control (24)	1.8 ± 0.3	2.38	6.2 ± 0.3	17.13^a	1.8 ± 0.3	6.2 ± 0.3	17.13^a
	+ Biotin (21)	1.8 ± 0.3	1.97	5.9 ± 0.3	15.28^a	1.8 ± 0.3	5.9 ± 0.3	15.28^a

Note: Figures in parentheses indicate sample size.

Appendices 3 to 11 provide further information of the observations of incidence of hoof defects and lesions not detailed in Tables 26 to 31.

[1] Differences between treatments non-significant over whole period of examination (Chi-square analysis).

[2] Differences between treatments statistically significant over whole period of examination (p <0.001; Chi-square analysis).

Differences between treatments statistically significant for single period of examination for each parameter (Chi-square analysis):

[a] p <0.05

[b] p <0.01

[c] p <0.001.

[d] Differences between treatments statistically significant for single period of examination for each parameter (p <0.05; one-way analysis of variance).

TABLE 27

Effect of dietary biotin supplementation and age of sow on mean number
of type of defect for each sow

Period of examination	Treatment	Bruising and abrasion				Cut
		Heel [1]	Heel/toe	Toe [2]	Side-wall [2]	Heel [3]
170 days	Control (38)	0.16 a	0.58	0.53	0.11	0.55
	+ Biotin (39)	0.47 c	0.44	0.41	d 0.03	0.33
First weaning	Control (36)	0.47 b	0.89	0.69	d 0.11	0.50
	+ Biotin (32)	0.84 b	0.66	1.16	0.13	0.34
Second weaning	Control (31)	0.52 b	0.84	0.87	0.16	0.32
	+ Biotin (29)	1.00 b	0.74	0.96	0.07	0.41
Third weaning	Control (27)	0.63 a	0.74	0.67	0.04	0.19
	+ Biotin (22)	0.26 a	0.70	0.48	0.04	0.26
Fourth weaning	Control (24)	0.71 a	0.75	0.38	0.04	0.04
	+ Biotin (21)	0.33 a	0.67	0.43	0.19	0.33

Note: Figures in parentheses indicate sample size.

All analysis by Chi-square.

[1] Differences between treatments statistically significant over whole period of examination ($p < 0.001$).

[2] Too and side-wall data amalgamated for analysis as insufficient data were present from the side-wall region for Chi-square analysis. Differences between treatments statistically significant for amalgamated data over whole period of examination ($p < 0.01$).

[3] Third and fourth weaning data amalgamated as insufficient data were present from the control treatment for Chi-square analysis. Differences between treatments statistically significant over whole period of examination ($p < 0.001$).

Differences between treatments statistically significant for single period of examination for each parameter:

[a] $p < 0.05$

[b] $p < 0.01$

[c] $p < 0.001$.

[d] Differences between treatments statistically significant for amalgamated toe and side-wall data for single period of examination ($p < 0.01$).

[o] Differences between treatments statistically significant for amalgamated third and fourth parity data for heel cuts ($p < 0.001$).

TABLE 28

Effect of dietary biotin supplementation and age of sow on mean number

of type of lesion for each sow

| Period of examination | Treatment | Lesion | | | | | Overgrowth |
| | | Erosion and crack | | | Crack | | |
		Heel[1]	Heel/toe	Toe[1] (median)	Toe[1] (white-line)	Side-wall[1]	Heel
170 days	Control (38)	0.03	0.39	0.08	2.47[a]	2.74[a]	0.00
	+ Biotin (39)	0.05	0.21	0.15	3.00[a]	2.08[a]	0.00
First weaning	Control (36)	1.08[c]	2.83	0.89	5.00[b]	4.17[c]	0.03
	+ Biotin (32)	0.47[c]	2.47	0.69	3.81[b]	2.41[c]	0.00
Second weaning	Control (31)	1.42	5.26	1.32[b]	4.13[b]	2.94	0.03
	+ Biotin (29)	1.00	5.07	0.72[b]	3.10[b]	2.97	0.03
Third weaning	Control (27)	2.37[c]	6.67	1.48[b]	4.44[b]	3.07	0.11
	+ Biotin (22)	1.27[c]	6.36	0.68[b]	3.18[b]	2.93	0.27
Fourth weaning	Control (24)	1.75	5.75	1.04	4.88[b]	3.96	0.00
	+ Biotin (21)	1.62	5.95	0.90	3.43[b]	3.38	0.00

Note: Figures in parentheses indicate sample size.

All analysis by Chi-square.

[1] Differences between treatments statistically significant over whole period of examination for each region ($p < 0.001$).

Differences between treatments statistically significant for single period of examination for each region:

[a] $p < 0.05$

[b] $p < 0.01$

[c] $p < 0.001$.

TABLE 29

Effect of dietary biotin supplementation and age of sow
on the mean number of lesions/claw on the outer and inner
claws of the fore and hind feet for all sows

Period of examination	Claw	Treatment		Degree of significance between treatments for each claw
		Control	+ Biotin	
170 days	Fore outer	0.83^a	0.86^a	
	Fore inner	0.38^{ac}	0.45^a	
	Hind outer	1.03^b	1.00^b	
	Hind inner	0.66^{bc}	0.45^b	*
First weaning	Fore outer	1.93^{ac}	1.44^{ac}	* *
	Fore inner	1.38^{ad}	0.91^{ad}	* *
	Hind outer	2.75^{bc}	2.02^{bc}	* * *
	Hind inner	0.66^{bd}	0.53^{bd}	
Second weaning	Fore outer	1.98^b	1.50^b	* *
	Fore inner	1.66^c	1.52^c	
	Hind outer	3.16^{ab}	2.90^{ab}	
	Hind inner	0.69^{ac}	0.57^{ac}	
Third weaning	Fore outer	2.19^b	1.89^{ac}	
	Fore inner	2.26^c	1.90^{ad}	* * *
	Hind outer	3.56^{ab}	3.05^{bc}	
	Hind inner	0.98^{ac}	0.77^{bd}	
Fourth weaning	Fore outer	1.83^b	1.86^b	
	Fore inner	2.04^c	1.57^c	*
	Hind outer	3.69^{ab}	3.31^{ab}	
	Hind inner	1.00^{ac}	0.91^{ac}	
Degree of significance between treatments for each claw over all periods	Fore outer	* *		
	Fore inner	* * *		
	Hind outer	* * *		
	Hind inner	*		

Note: 1) Analysis by Chi-square.

2) Statistically significant differences were observed for the values of each claw analysed over all periods of examination within the control and biotin supplemented treatments respectively (p < 0.05 or greater).

3) Values with the same superscript within a treatment and a period of examination differ statistically significantly (p < 0.05 or greater).

FIGURE 17

Percentage distribution of sows for the number of claws with defects

i. At 170 days

ii. At first weaning

Control treatment

+ Biotin treatment
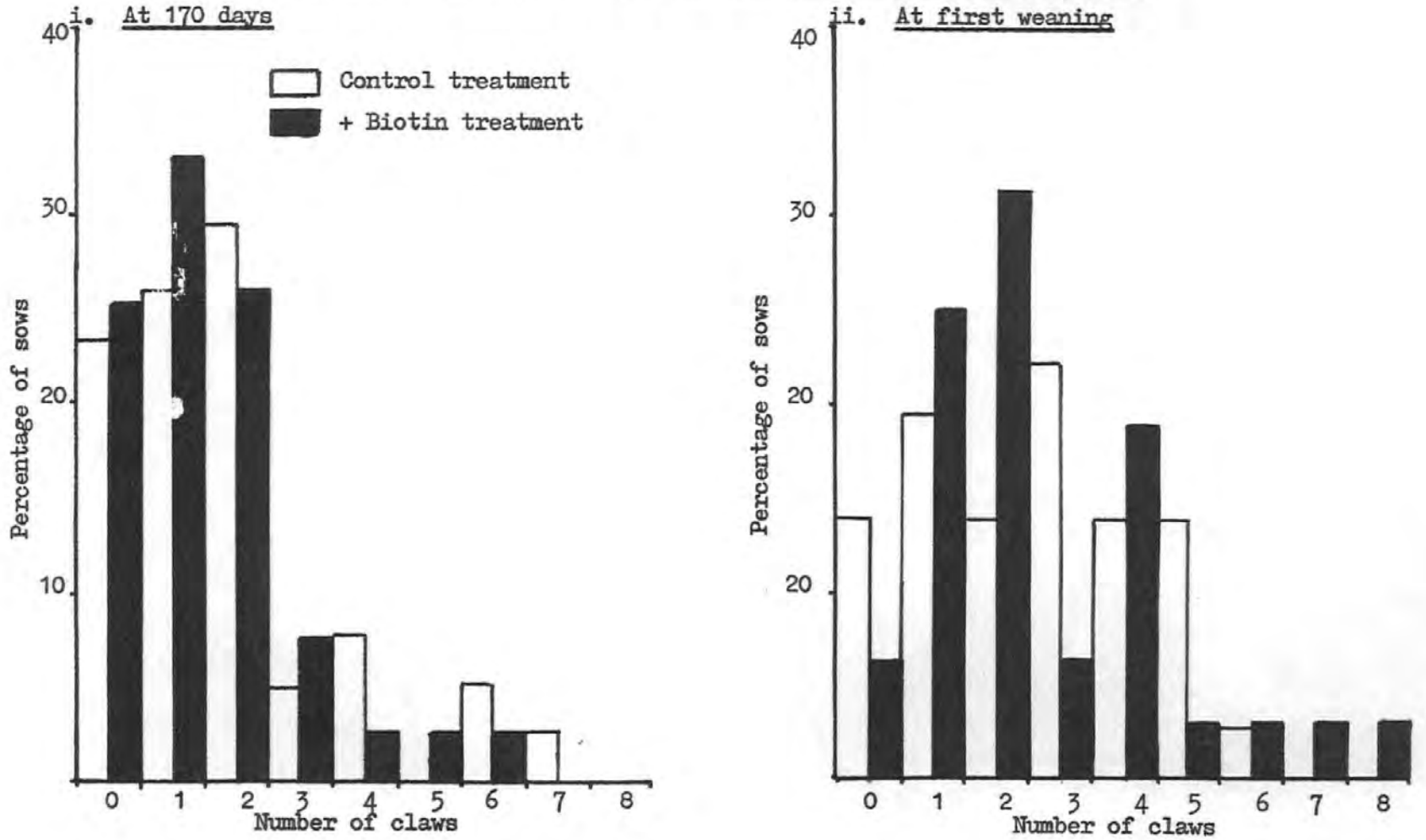
Note: Data presented in Appendix 3.

FIGURE 18

Percentage distribution of sows for the number of claws with defects

i. At second weaning

ii. At third weaning

Control treatment

+ Biotin treatment

Percentage of sows
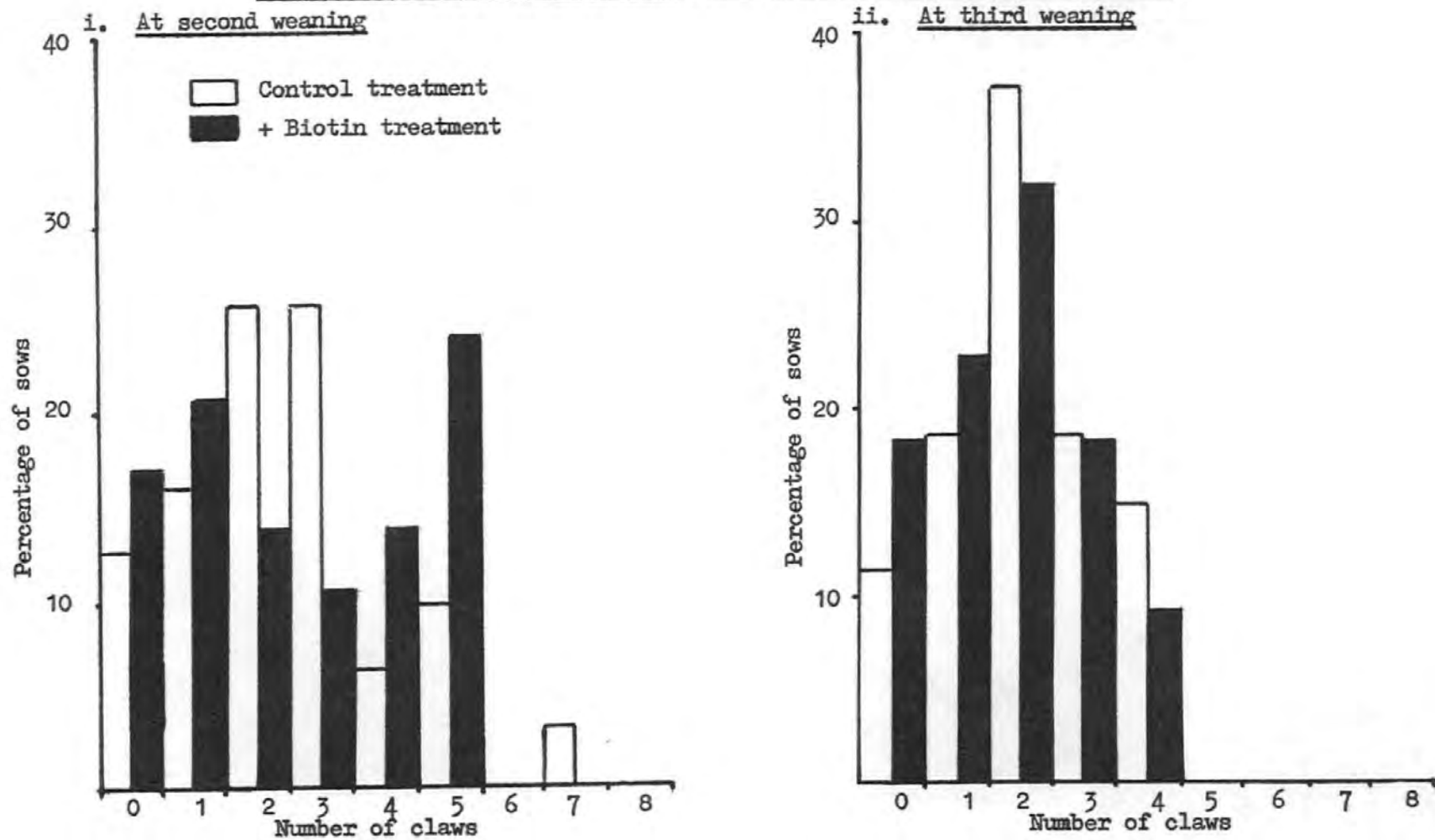
Number of claws

Note: Data presented in Appendix 3.

FIGURE 19

Percentage distribution of sows for
the number of claws with defects

At fourth weaning

Control treatment

+ Biotin treatment
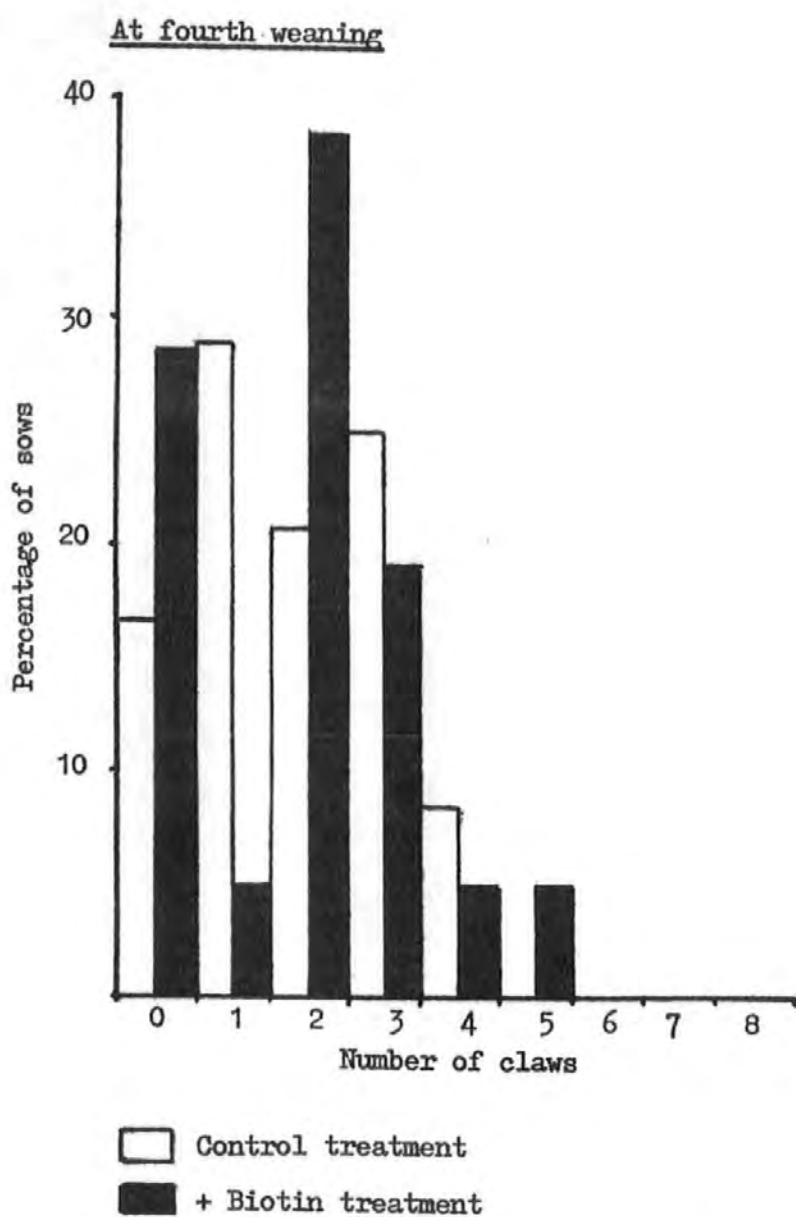
Note: Data presented in Appendix 3.

FIGURE 20

Percentage distribution of sows for the number of claws with lesions

i. At 170 days

ii. At first weaning

☐ Control treatment

■ + Biotin treatment

Percentage of sows

Number of claws

Percentage of sows
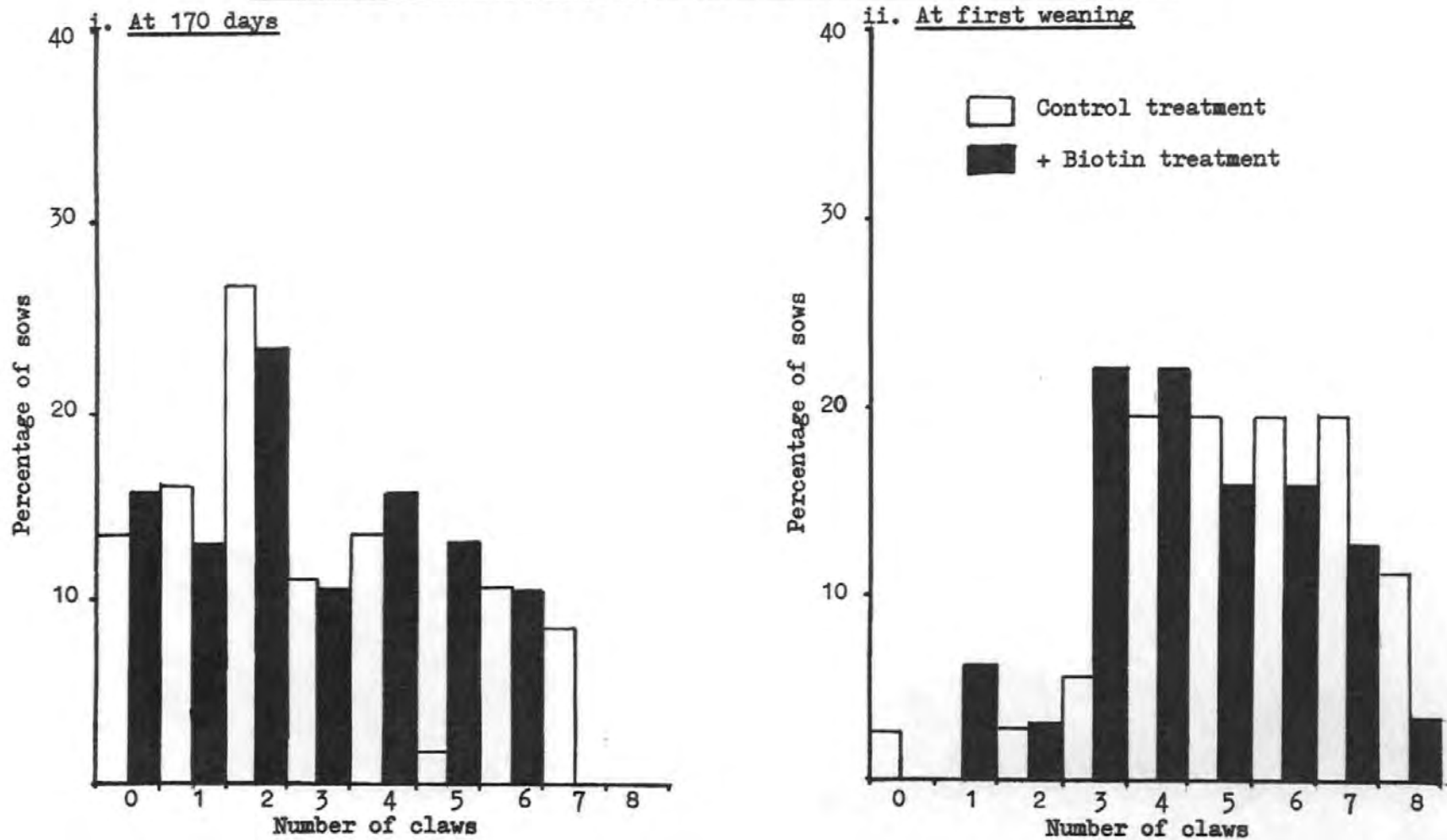
Number of claws

Note: Data presented in Appendix 6.

FIGURE 21

Percentage distribution of sows for the number of claws with lesions

i. At second weaning

ii At third weaning

Control treatment

+ Biotin treatment

Percentage of sows

Number of claws

Percentage of sows

Number of claws

82
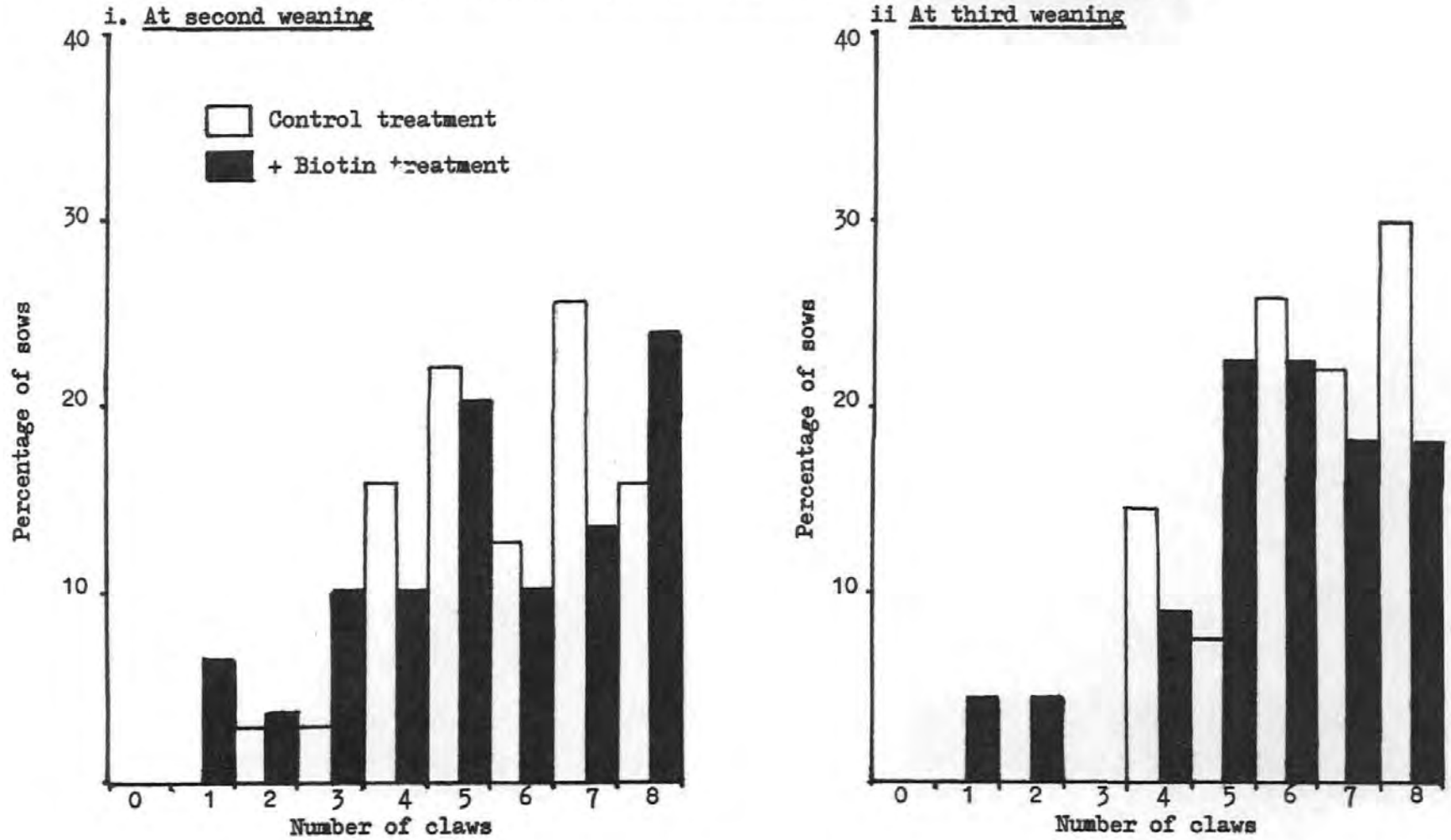
Note: Data presented in Appendix 6.

FIGURE 22

Percentage distribution of sows for
the number of claws with lesions

At fourth weaning

Control treatment

+ Biotin treatment
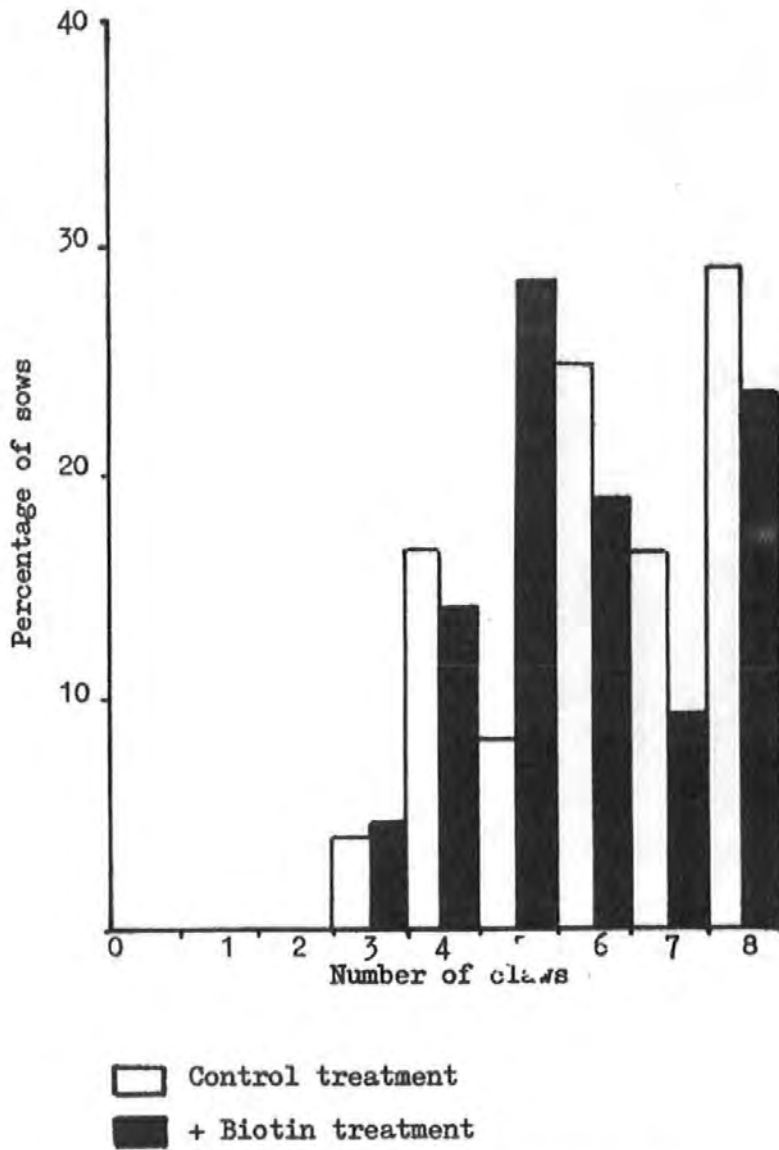
Note: Data presented in Appendix 6.

2. Observations at first weaning

The hooves of both groups of pigs had deteriorated by first weaning.
2.6 ± 0.3 claws were affected by defects in both treatment groups at a
rate of 2.97 and 3.38 defects/sow for the control and supplemented
treatments respectively (Table 26). The supplemented group had sig-
nificantly more defects than the control group for the heel and
combined toe and side-wall regions ($p < 0.05$, Table 27).

Lesions were observed on 5.4 ± 0.3 of claws in the control group and
4.5 ± 0.3 of claws in the supplemented group (Table 26). This resulted
in significantly more claws being affected by lesions in the control
group (1.1 ± 0.3; $p < 0.05$). Associated with this, the number of
lesions/sow had increased to a greater extent in the control group
($c = 13.4$ vs $s = 9.8$; $p < 0.001$). There was an increase in the number
of lesions in the heel/toe region in both treatment groups as a result
of the appearance of erosions mainly at the axial heel/toe junction
(Table 28). In some cases such erosion appeared to have developed into
cracks extending to the abaxial edge resulting in separation of the
heel and toe. Rarely did a heel/toe crack exist without accompanying
erosion, therefore it was not practicable to differentiate these two
lesions. Similarly, both abaxial and axial lesions at the heel/toe
junction were assessed together as the former was usually associated
with the latter. Cracks that were identified in the heel region alone
were less in number than at the heel/toe junction, but significantly
more were recorded for the control compared to the supplemented
treatment ($p < 0.001$).

Cracks in the white line of the toe and on the side-wall had increased
in both treatments. It was the greater incidence of these lesions in
the control group ($c = 9.14$ vs $s = 6.22$; $p < 0.01$) which was mainly
responsible for the statistically significant difference in the mean
number of lesions/treatment. Some lateral cracks were still "hairline"
but others were wide and deep with obvious sub-surface haemorrhaging.
Horizontal cracks were often associated with vertical cracks and may
have originated where a vertical crack erupted at the coronet. In some
instances this led to a series of two or three horizontal cracks at
intervals along the vertical crack which appeared to be growing out away
from the coronet to the volar edge. The abaxial white line lesions were

often associated with vertical lesions. These were also linked to sub-surface vertical lesions, which although clearly visible, did not break the horn surface but could extend upwards to and erupt at the coronet as a horizontal crack.

Very few sows had claws without lesions and a greater proportion had seven or eight claws affected (Figure 20). The fore outer and inner and hind outer claws of the supplemented sows showed a consistently lower proportion of lesions than those of the control sows (p◁0.01 or greater; Table 29). Within treatments, the inner claws were the least damaged (p◁0.05 or greater) and the hind outer claws showed most damage from lesions, having significantly more lesions than even the fore outer claws (p◁0.05 or greater).

3.   Observations at second weaning

By second weaning the number of claws/sow with defects and the level of defects/sow had remained unchanged, but the condition of hooves had deteriorated for both treatments which was shown by the increase in the mean number of claws affected by lesions (c = 5.8 $\pm$ 0.3 vs s = 5.4 $\pm$ 0.4; Table 26). The total number of lesions/sow had increased at this time with significantly more lesions/sow observed in the control group (c = 15.0; s = 13.0; p◁0.01). The increase from the previous weaning was mainly due to a higher number of lesions in the heel/toe region (c = 2.8 and 5.3 vs s = 2.5 and 5.1 at first and second weaning respectively, Table 28). Over the same period the number of cracks in the white line and side-wall regions had decreased in the control group but increased slightly in the supplemented group. However, significantly more lesions were still noted in the median and white line regions of the toe respectively for the control treatment (p◁0.01 for both regions).

No sows were without any lesions and a greater proportion had seven or eight claws with lesions (Figure 21). The inner and outer claws of the control sows had more lesions that the supplemented sows (statistically significant only for the fore outer claws at p◁0.01). Within treatments the outer claws of the hind feet were the most seriously damaged compared to the other claws (p◁0.001). In contrast the inner claws of the hind feet had the least number of lesions/sow

compared to the other claws (p ◁0.001) and showed little change from
the previous examination. The inner and outer claws of the front feet
exhibited an intermediate and similar level of damage.

4. Observations at third weaning

Although a slight reduction in the number of claws/sow with defects and
the level of defects was observed in both treatments, the increase in
the number of claws having lesions was maintained (c = 6.4 $\pm$ 0.3 vs
s = 5.7 $\pm$ 0.4; Table 26). There was a further increase in the number
of lesions per sow in both groups (c = 18.0 vs s = 14.4; p ◁0.001).
This was due mainly to a further increase in heel/toe and heel lesions
in both treatment groups (Table 28). The control sows also showed
significantly more lesions than the supplemented sows in the heel
regions (p ◁0.001). Generally, continued deterioration of the soft
heel appeared to have resulted from the extension of axial heel/toe
lesions, not only to the abaxial region of the heel/toe junction but also
into the heel bulb. Abaxial white line cracks in the toe and vertical
side-wall cracks were still present in both treatments, with significan-
tly more lesions in the median and white-line regions of the toe
observed in the control sows compared to the supplemented sows
(p ◁0.01 for both regions).

All the control sows had lesions on at least four claws and 51.8% of
these sows had seven or eight claws with lesions. The supplemented
group showed a greater variation with only 36.4% of sows having seven
or eight claws with lesions. The distribution of lesions between
individual claws resulted in the control group having more lesions on
the inner and outer claws than the respective claws of the supplemented
group (statistically significant only for the fore inner claws at p◁0.001;
Table 29). However the distribution of lesions between the inner and
outer claws of the fore and hind feet remained similar to that observed
at second weaning within each treatment group.

5. Observations at fourth weaning
Again, the number of defects was maintained at a similar level to
previous examinations (Table 26). However the treatment differences
for lesion damage were less apparent at fourth weaning. A reduction in
the number of claws/sow with lesions was observed in the control group
whereas a slight increase was noted in the supplemented group (c = 6.4

86

$\pm$ 0.5 and 5.7 $\pm$ 0.4 vs s = 6.2 $\pm$ 0.3 and 5.9 $\pm$ 0.3 for the third and
fourth weanings respectively). The number of lesions/sow also declined
in the sows on the control treatment (17.1/sow)and increased in the case
of the supplemented sows (15.3/sow) but the difference between treat-
ments was still statistically significant ($p < 0.05$). This was partly as
a result of a reduction of the number of lesions in the heel and heel/
toe regions in the control sows, although there was a further minor
deterioration in the supplemented sows (c = 9.0 and 7.5 vs s = 7.3 and
7.6 lesions/sow for the combined heel/toe and heel regions at third and
fourth weanings respectively; Table 28). In addition, the white line
region of the toe and the side-wall in both groups exhibited further
deterioration, the number of lesions in the white line being still
significantly greater for the control treatment ($p < 0.01$).

As before, more control sows had seven or eight claws with lesions
(45.9%) than the supplemented sows (33.3%) but the incidence was lower
than on the previous assessment for both treatments (Figure 22). The
differences in the number of lesions between individual claws of the
control and supplemented sows was generally less, but as previously, the
fore inner claw showed significantly more lesions in the control group
than the supplemented group ($p < 0.05$). The distribution of lesions
within treatments between claws remained similar to earlier observations.

6. <u>Changes in distribution of defects and lesions with age of the sow</u>
Defects remained at a low level compared to lesions throughout the Trial.
A small increase was observed in the number of claws with defects and
defects/sow between 170 days and first weaning for both treatments
(Figure 23). Subsequently a slight reduction in these parameters
occurred with age; the differences between treatments were not
statistically significant.

The distribution of defects between claws was generally similar at 170
days and for most observations (Appendix 4). Some exceptions were
observed, with the hind outer claws having a tendency to more defects
than the hind inner claws from first weaning, whereas the fore claws
showed a variable response with age. The supplemented treatment at
first and second weaning and the control treatment at second weaning
only, had slightly more defects on the fore outer compared to the
fore inner claws. The major defects were bruising and abrasion of the

FIGURE 23

Effect of treatment and parity on the distribution of i) claws per sow with defects and lesions, and ii) defects and lesions per sow
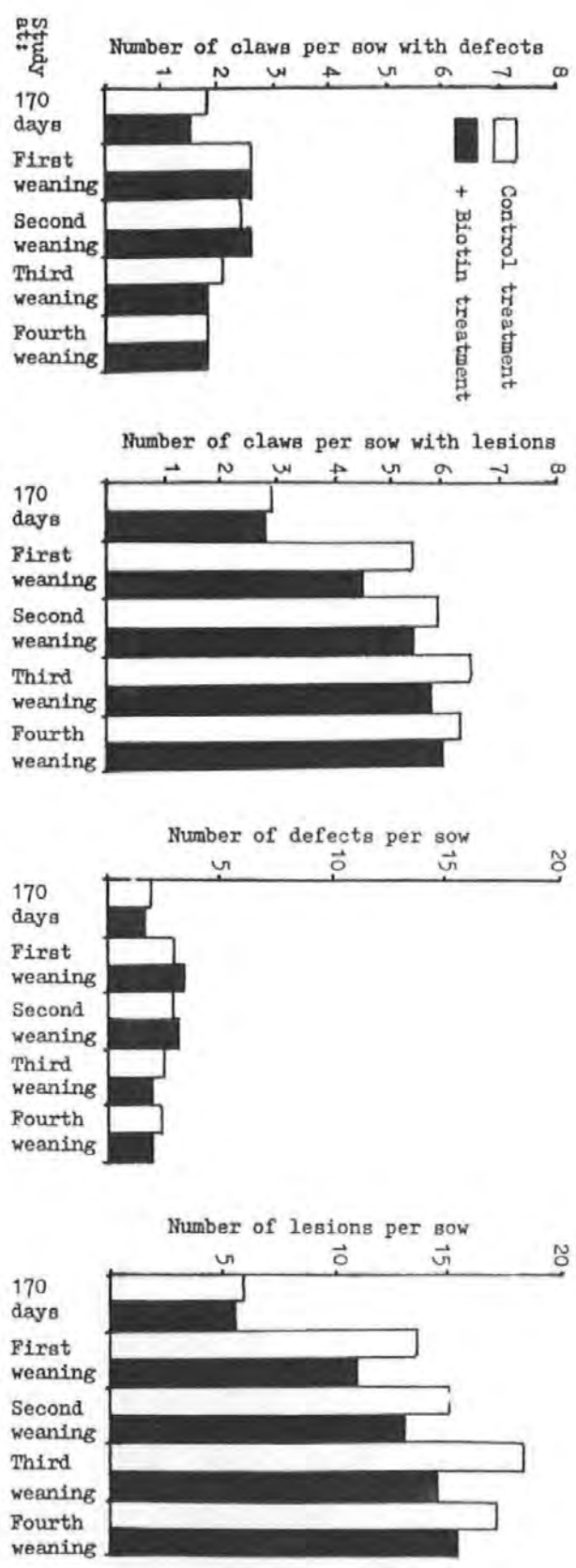
FIGURE 24

Effect of treatment and parity on the distribution of lesions/sow for the
inner and outer claws of the fore and hind feet
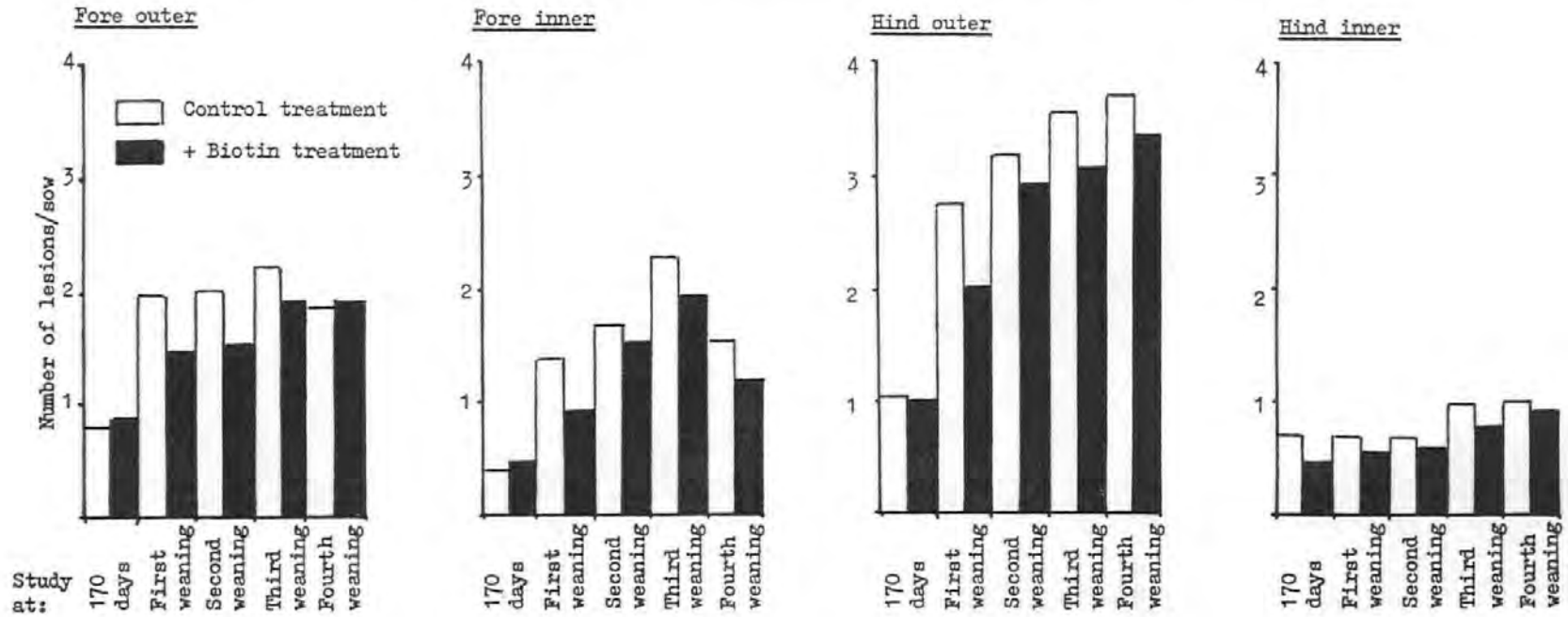
heel/toe and heel regions combined, which showed a consistent small
increase in the control sows throughout the trial but reached a
maximum at second weaning in the supplemented group (Table 27). In
fact, up to and including second weaning, significantly more heel
defects were observed in the supplemented sows compared to the control
sows (p ⊲0.01 or greater for each observation), whereas for the third
and fourth weanings the control sows displayed significantly more
defects in the heel than the supplemented sows (p ⊲0.05 for each
observation). The toe region primarily suffered from abrasions. The
supplemented sows showed a large increase in this defect between 170
days and first weaning, which when combined with the results from the
few side-wall defects gave statistically significantly more defects
for the supplemented compared to the control group (p ⊲0.01). Sub-
sequently, the numbers greatly reduced in the supplemented sows with
successive observations. The control group showed a similar trend
but peaked at the second weaning. Cuts, present on the heel only,
showed a gradual reduction with age in the control group, remaining at
a constant level in the supplemented group. The third and fourth
weaning data, when combined for analysis, showed significantly more
cuts in the supplemented compared to the control sows (p ⊲0.001). The
side-wall exhibited little injury from defects.

The number of lesions/sow greatly increased between 170 days and first
weaning when they contributed to the major proportion of the total
injury to the hoof (Figure 23). As stated earlier, significant
differences between treatments were also first observed at first weaning
with more lesions/sow and claws/sow with lesions in the control sows
compared to the supplemented sows (p ⊲0.001 and p ⊲0.05 respectively).
The maximum number of lesions/sow was achieved at third weaning in the
control group and at fourth weaning in the supplemented group.
Differences between readings were progressively smaller for the
supplemented treatment and it appeared that the number of lesions/sow
was stabilising in the oldest sows. A similar trend was also displayed
by the mean number of claws affected by lesions for both treatments
(Figure 21).

The fore and hind outer claws were more damaged by lesions than the
fore and hind inner claws for both treatments at the 170 day

observation (Table 29 and Figure 24). By first weaning, the hind outer claws showed a much greater degree of injury than the fore outer claws. The fore inner claws displayed an increase in injury whereas the level of damage to the hind inner claws was maintained. The measurements at fourth weaning confirmed that the outer hind claws were the most prone to injury and the inner hind claws the least. The inner and outer claws of the fore feet tended to show damage at a level intermediate to those of the inner and outer claws of the hind feet. Overall, a significant difference was observed for each claw between treatments although the hind inner claws contributed least to the treatment differences. Most contribution to the treatment differences was displayed by the fore inner and hind outer claws. Both, the increase in overall number of hoof lesions between 170 days and first weaning, and the significant differences between treatments at first weaning, were displayed by the individual claws except for the hind inner claws. A statistically significantly greater number of lesions/claw was shown by the other claws individually for the control compared to the supplemented treatment (p ⊲0.01 or greater).

Injury due to lesions appeared mainly in two associated areas: the heel/ toe and heel; the side-wall and white line region of the toe (Table 28). Cracks in the latter were primarily responsible for the increase in lesions between the 170 day and first weaning observations. By second weaning, further increase in lesions/sow was due mainly to cracks and erosions in the heel/toe region. These extended into the heel bulb by third weaning. The fourth weaning assessment showed some improvement in these lesions which highlighted the further deterioration in the white line and side-wall cracks. Over time there had been a small increase in the level of injury to the median region of the toe. Much of this was due to large areas of damage which linked lesions at the heel/toe junction to those at the white line and side-wall regions. Overgrowth lesions were always a minor proportion of the total lesions examined.

7. Observations on sows completing four parities

The results for only those sows completing four parities were compared to the analysis for all sows.

The mean number of claws/sow with defects had remained unchanged with age and treatment for sows completing four parities (Table 26). Further investigation of defects on these sows was not undertaken as defects remained at too low a level to provide sufficient data to show possible treatment differences.

However previous significant treatment effects on lesions merited further investigation. The same trend was apparent in the number of claws/sow with lesions and incidence of lesions/sow for all sows and for those sows completing four parities. The difference between treatments at 170 days of age for lesions/sow was significant statistically (c = 6.2 vs s = 4.9; p <0.05), whereas no statistically significant difference was observed on the data for all sows. The distribution of type of lesion/region/sow also remained similar with age and treatment (Table 30). Data for the heel and heel/toe regions were combined for analysis which showed that significantly more lesions were present in the control sows compared to the supplemented sows at 170 days, first and fourth weaning (p <0.05 or greater).

The effects of treatment on individual claws over all observations and at each observation displayed a similar trend to that recorded for all sows, although differences in levels of statistical significance occurred for sows completing four parities. Similar effects were shown within treatments at each observation. It is noteworthy that each claw category showed a significantly higher incidence of lesions at first weaning in the control group compared to the supplemented group (p <0.05 or greater). The differences between treatments for the hind inner claws was not statistically significant at this observation for all sows (Table 31).

DISCUSSION AND CONCLUSIONS

Assays

It is not clear why the microbiological assay technique should record positive, albeit small, values for the vitamin premixes without biotin added. Conversely the premixes with a theoretical 350 µg/kg biotin added, recorded values considerably lower than this. The latter point may be explained by a loss of biological activity from the added biotin

92

TABLE 30

Effect of dietary biotin supplementation and age of sow on mean number
of type of lesion for each sow completing four parities

Period of examination	Treatment	Lesion					Overgrowth
		Erosion and crack			Crack		
		Heel[1]	Heel/toe[1]	Toe[2] (median)	Toe[3] (white-line)	Side-wall[3]	Heel
170 days	Control (24)	0.04	0.50 a	0.08	2.79	2.75 d	0.00
	+ Biotin (21)	0.00	0.14 a	0.14	2.95	1.95 d	0.00
First weaning	Control (24)	0.58	2.54 b	0.83	5.08 d	4.08 f	0.04
	+ Biotin (21)	0.19	1.52 b	0.76	3.90 d	1.95 f	0.00
Second weaning	Control (24)	1.17	4.92	1.33 d	4.63 e	3.04	0.00
	+ Biotin (21)	0.90	5.14	0.67 d	3.19 e	3.10	0.05
Third weaning	Control (24)	2.42	6.21	1.50 e	4.88 f	3.38 d	0.13
	+ Biotin (21)	1.29	6.38	0.71 e	3.05 f	2.48 d	0.29
Fourth weaning	Control (24)	1.75	5.75 c	1.04	4.88 e	3.96	0.00
	+ Biotin (21)	1.62	5.95 c	0.90	3.43 e	3.38	0.00

Note: Figures in parentheses indicate sample size.

Analysis by Chi-square.

[1] Heel and heel/toe data combined for analysis.
Differences between treatments statistically significant over whole period of examination ($p < 0.001$).
Differences between treatments statistically significant over whole period of examination for each region:

[2] $p < 0.01$

[3] $p < 0.001$.

Differences between treatments statistically significant for single period of examination for combined heel and heel/toe regions:

[a] $p < 0.05$

[b] $p < 0.01$

[c] $p < 0.001$.

Differences between treatments statistically significant for single period of examination for each region:

[d] $p < 0.05$

[e] $p < 0.01$

[f] $p < 0.001$.

TABLE 31

Effect of dietary biotin supplementation and age of sow on the
mean number of lesions/claw on the outer and inner claws of the
fore and hind feet for sows completing four parities

Period of examination	Claw	Treatment		Degree of significance between treatments for each claw
		Control	+ Biotin	
170 days	Fore outer	0.81	0.67^a	
	Fore inner	0.35^a	0.33^a	
	Hind outer	1.08	0.98^b	
	Hind inner	0.85^a	0.48^b	* *
First weaning	Fore outer	1.77^b	0.95^b	* * *
	Fore inner	1.56^c	0.90^c	* * *
	Hind outer	2.60^{ab}	1.93^{ab}	* *
	Hind inner	0.69^{ac}	0.40^{ac}	*
Second weaning	Fore outer	1.88^b	1.48^b	
	Fore inner	1.75^c	1.74^c	
	Hind outer	3.23^{ab}	2.81^{ab}	
	Hind inner	0.77^{ac}	0.55^{ac}	
Third weaning	Fore outer	2.19^b	1.91^{ac}	
	Fore inner	2.31^c	1.52^{ad}	* * *
	Hind outer	3.65^{ab}	3.00^{bc}	*
	Hind inner	1.02^{ac}	0.67^{bd}	*
Fourth weaning	Fore outer	1.83^b	1.86^b	
	Fore inner	2.04^c	1.57^c	*
	Hind outer	3.69^{ab}	3.31^{ab}	
	Hind inner	1.00^{ac}	0.91^{ac}	
Degree of significance between treatments for each claw over all periods	Fore outer	* * *		
	Fore inner	* * *		
	Hind outer	* *		
	Hind inner	* *		

Note: 1) Analysis by Chi-square.

2) Statistically significant differences were observed for the values of each claw analysed over all periods of examination within the control and biotin supplemented treatments respectively ($p < 0.01$ or greater).

3) Values with the same superscript within a treatment and a period of examination differ statistically significantly ($p < 0.05$ or greater).

by the time the assay was undertaken. It is understood that the biological activity of naturally occurring biotin derivatives may differ when determined microbiologically (Frigg, 1976). Hence the variation in the basal diets from 29 to 155 µg available biotin/kg could be quite acceptable when considering the feedstuff alone. The results from the premix analyses suggest that further measurable variation occurs from the assay technique itself, hence results of plasma biotin assays by this technique on the individual sow should be treated with caution.

The lowest values recorded for the biotin content of the plasma were from the control sows which were close to the level of 40 ng/100 ml which Tagwerker (1973) suggested would indicate biotin deficiency. Therefore the unsupplemented diet, in providing 32 µg of estimated available biotin/kg, resulted in an inadequate intake of dietary biotin. The slight reduction in plasma biotin levels from parturition to weaning corresponded with observations by P H Brooks (1977, personal communication). This may be explained by the high metabolic demands of lactation, when considerable quantities of biotin are required to support fat synthesis and fat secretion in the milk. Supplementing the diets with a theorectical 350 µg biotin/kg resulted in higher plasma biotin levels and these were maintained throughout the period of lactation.

Reproductive performance

The effect of supplementary dietary biotin on sow reproduction manifested itself after weaning of the first litter. Penny et al. (1981) achieved a similar result but other workers have found a positive effect on gilt performance. A non-statistically significant improvement from 8.5 to 9.2 pigs born and from 7.4 to 8.5 pigs weaned in gilt litter size was reported by Easter et al. (1979). The gilts in their trial were fed a corn/soya diet supplemented with 200 µg biotin/kg.

Lactation is known to result in a drain on the energy reserves of a gilt and it appears that the level of dietary biotin in a normal diet may be insufficient in lactation and the period directly following weaning. Prior to the first lactation, the gilts were less likely to have had high metabolic demands suggesting that the biotin requirements of both

groups were low during this period. In fact the litter size and weight at birth were non-significantly greater in the control treatment for the first litter. However, by weaning, the litter weight was similar for both treatments. Subsequently, overall productivity favoured the litters of supplemented sows in both number and overall weight of piglets. The work of Robres Serrano and Garcia de la Calera (1981) also showed an improvement in litter size and live-weight following biotin supplementation.

The effect of dietary biotin supplementation on performance was almost certainly underestimated in this experiment as the herd practice was to induce oestrus within 29 days of weaning. In fact, more control than supplemented sows were successfully treated to induce oestrus. This would indicate that without such treatment more control sows would have been culled due to anoestrus and the interval from weaning to oestrus and conception would have been increased compared with the supplemented treatment.

The finding that biotin supplementation reduces weaning to oestrus interval and improves conception rate confirmed the earlier results obtained at the same centre (Brooks et al., 1977). These effects have since been confirmed by other workers (Halama, 1979; Pedersen and Udesen, 1980; Bryant et al., 1981).

Hoof studies

The increase in hoof damage was related to the age of the sow. Defects remained at a similar level throughout the life of the sows, so as excitory factors in lesion formation their effect remained reasonably constant with time. It appeared that the initial rate of damage did not substantially change but repair of the hoof was occurring at a slower rate as the sow aged. The large increase in lesions between the observations at 170 days and at first weaning indicate that by the latter stage the condition of the hoof horn was mainly influenced by lesions. However the development of defects into lesions was higher in the control group at the first, second and third weanings, so it may have been expected that more defects would have been observed in the supplemented sows over these periods. There was some evidence for this, but judgement of damage by defects was also more difficult to assess in

96

both treatment groups, as the incidence of lesions increased with age. That the control sows exhibited significantly more claws with lesions by first weaning also further confirms the effects of the first lactation on the biotin demands of the young sow. Triebel and Lobsiger (1979) and De Jong and Sytsema (1983) interpreted this as a preventative effect of biotin supplementation on the development of claw lesions.

The number of lesions/claw appeared to be stabilising in the older sows and the treatment differences were less. Penny et al. (1980) observed that biotin supplementation was of less value as a treatment for established foot lesions. These results, as well as similar ones for the reproductive performance, suggest that the level of dietary biotin plays an important role in the health of the young sow. She is a growing animal and may have a higher demand for biotin than the mature sow. As a result the young sow may be more likely to enter periods of biotin deficiency at certain times in the breeding cycle, particularly lactation.

The hind outer claws were most prone to injury and the hind inner the least. Penny et al. (1965) in their survey of slaughtered bacon weight pigs, noted that ratio of lesions was at least 4:3 for the fore outer and inner claws and $3\frac{1}{2}$:1 for the hind outer and inner claws. This is due to the inequality in size of digits, which is least in the fore feet, so appear to share more in the weight-bearing load. Nordby (1939) originally pointed out the inequality of digits in swine with the outer claws being larger than the inner. He also stated that the unequal sizes distorted the phalanges which altered the gait and caused lameness. More recently, Geyer (1979) has reported that the area of weight-bearing is greater on the outer claws which further confirms that the outer claws would be more prone to injury. Arthur et al. (1983) have also noted that the outer claws generally showed more lesions than the inner, with the hind outer displaying the greatest number of lesions. In experiment 1, biotin supplementation did not prevent these trends but it did reduce the severity of damage to the hooves. Penny et al. (1980) also showed that dietary biotin supplementation resulted in a statistically significant reduction in the number of lesions in the hind outer claws. However biotin supplementation has also reduced the number

97

of lesions, mainly on the inner claws (Brooks et al., 1977) and the hind and inner claws (Grandhi and Strain, 1980). It has been suggested by Tagwerker (1973), Brooks et al. (1977) and Penny et al. (1980) that the effect of biotin supplementation is to harden the hoof which may give added protection to the feet of the growing sow but is less significant once she has reached maturity when the sow's demand for biotin may not be as great.

The improvement in the condition of the hooves of the older sows was due to a regression of the lesions on the volar surface in both treatments. This was similar to the findings reported by Penny et al. (1980). Bujas et al. (1972, cited by Tagwerker, 1973) and Bryant et al. (1980) noted that a lessening in the severity of lesions associated with the soft heel occurred with biotin supplementation. Brooks et al. (1977) and Bryant et al. (1980) also observed a reduced incidence of heel/toe cracks with biotin supplementation. Brooks et al. (1977) further reported an increase in heel lesions with age of the sow, but the increase was reduced by biotin supplementation. An improvement following biotin supplementation was reported by De Jong and Sytsema (1983) not only on the volar surface but also on the side-wall, but to a lesser extent. Their trial had more side-wall lesions overall than other work.

Horizontal lesions occurred on the side-wall which were considered by Brooks et al. (1977) to be diagnostic of biotin deficiency as Penny et al. (1963) had not reported this type of lesion in his slaughterhouse survey. They occurred in both treatments in this experiment and whereas Brooks et al. (1977) could assume that most sows were biotin deficient on commencement of that experiment, this was not the case in the current study. However the white line of the toe provided statistically significantly more lesions in the control compared to the supplemented sows in every observation from and including first weaning. White line lesions are regularly associated with lesions in the side-wall and any challenge to the hoof horn of the side-wall may first reveal itself at the white line. It is noteworthy also that while the heel and heel/toe lesions were reduced in incidence at fourth weaning, the lesions of the white line and side-wall continued to increase in both treatments. It may be that these lesions are generally prevalent where the pig's hoof

horn faces a high degree of challenge from the environment and that an inadequate supply of dietary biotin results in the hoof horn being prone to continued damage in this area. Certainly, once a crack formed on the side-wall, secondary infection and under-running frequently followed which can lead to lameness. Although the level of culling was not influenced by treatment in this trial, De Jong and Sytsema (1983) have shown that the rate of culling through lameness can be considerably reduced in a commercial herd following dietary biotin supplementation.

The effects of dietary biotin supplementation on reproductive performance and hoof integrity must also be influenced by management, environment and other nutritional factors. Given these limitations, this experiment shows that supplementation of commercial diets with biotin provides the opportunity for the potential performance of the sow to be more nearly obtained. However this experiment does not permit any conclusions concerning the pathways through which biotin influences these parameters or the minimum level of dietary biotin necessary to achieve these results. Further investigation of some of these factors was undertaken in the following experiments.

EXPERIMENT TWO
THE EFFECT OF BIOTIN DEFICIENCY ON OVULATION
RATE AT THE SECOND OESTRUS PERIOD OF THE GILT

OBJECT

Depression in growth rate and changes in the integrity of the integument of the pig have followed the feeding of diets deficient in biotin (Tagwerker, 1973). When dietary biotin intake is at this level, reproduction, a complex metabolic activity, may also suffer. Brooks et al. (1977) first reported that the supplementation of commercial diets with biotin resulted in an improvement in litter size of second parity sows. These observations may be explained by an increase in ovulation rate. The object of this experiment was to study the effects of biotin supplementation on the ovulation rate of the gilt at the second heat period (the oestrus period during which gilts were served in experiment 1).

MATERIALS AND METHODS

Treatments

Two groups, each of 8 gilts, were fed from 24 hours after detection of first oestrus until slaughter eight days after the second oestrus period, either:

        A) basal diet plus 1% egg white; or

        B) basal diet plus supplementary biotin.

The gilts were weighed on allocation to the treatments, at puberty and at second oestrus. The age at puberty, second oestrus and at slaughter was recorded. Feed consumed was also recorded.

Diets

The formulation of the basal diet was to the same specification as Diet B of experiment 1 and was described in materials and methods of experiment 1 (Tables 10 and 11). In outline it contained 14.4% CP and

13.1 MJ DE/kg.

Treatment A contained, in addition, dried egg white, as a source of
avidin which is a biotin-binding agent. The inclusion of egg white at
1% was calculated to be sufficient to bind all the biotin present in
the other feed ingredients (Appendix 12). Treatment B was supplemented
with 35 g Rovimix H (Roche Products Ltd)/tonne which provided 350 μg
biotin/kg. This level was considerably above the recommended levels
and should have ensured biotin sufficiency.

Feeding

On allocation the gilts were fed a proprietary grower diet. At one
hundred and forty days of age both groups were transferred to the
biotin-supplemented diet, treatment B, and individually fed on an
increasing scale (Table 32). All food not consumed was weighed and
recorded. Twenty-four hours after the "standing period" at first heat
the two groups, each of eight pigs, were given treatments A and B
respectively at a rate of 3.0 kg/day until slaughter on or after the
eighth day following the second heat period.

The gilts were weighed on the Monday of each week and the ration
changed on the following Wednesday.

Housing

The gilts were housed in the rearing accommodation described in
experiment 1. They were placed in 2 pens each holding 4 animals from
each treatment. The gilts were in isolation from males until they were
170 days of age. They were then moved to sow yards where they had con-
tinuous aural, visual and olfactory contact with sexually mature,
intact boars.

Testing for oestrus was undertaken daily as described in experiment 1.

Examination of Reproductive Tracts

Reproductive tracts were removed immediately after slaughter. The
ovaries were examined when fresh and a preliminary count was made of the
corpora lutea resulting from the second oestrus period and the corpora
albicantia resulting from the first oestrus period. They were then
placed in formal-saline for at least 24 hours. This not only acted as

101

TABLE 32

Daily rations from 140 days of age until slaughter

Weeks on test	Ration kg/pig/day
1	2.3
2	2.5
3	2.7
4	2.9
5	3.0
Remains on	
3 kg/day	
until slaughter	

a preservative but it also hardened the tissues of the ovaries so that dissection could be facilitated and a second count made of the corpora lutea and corpora albicantia.

The ovaries were weighed prior to dissection.

The connective tissue was carefully removed from the horns of the uteri. The length of the left and right fallopian tubes and left and right uterine horns (measured from the uterotubal junction to the utero-cervical junction) was measured.

RESULTS

One gilt in treatment A received a severe shoulder injury and was slaughtered prior to puberty. Therefore the results for only seven gilts from treatment A were analysed.

Treatment differences were analysed using a one-way analysis of variance. The analyses showed no significant difference between the parameters studied. The small sample size would make statistically significant results difficult to attain.

The biotin-supplemented gilts (treatment B) were slightly heavier at the start of the experiment and they maintained the slight advantage in weight throughout the trial. In fact, the growth rate of treatment B was 800 g/day compared to 758 g/day (s.e.d. $\pm$ 153 g/day) for the biotin-deficient treatment, a 5.3% faster growth rate for the supplemented gilts (Table 33). The period from introduction to the boar and puberty was similar in both treatments, 8.6 and 8.9 (s.e.d. $\pm$ 1.3) for the biotin-deficient and biotin-supplemented treatments respectively.

The left and right ovaries of both treatments produced a total of 14 $\pm$ 0.5 corpora lutea from the second heat period (Table 34).

Although the weights of the biotin-supplemented ovaries were only marginally higher, the trend in the length of the reproductive tracts indicated that the biotin-deficient gilts were smaller by 16.4 and 17.1% for the right and left uterine horns respectively (Table 35).

103

TABLE 33

Performance parameters of treatments

Parameter	Biotin-deficient treatment A	Biotin-supplemented treatment B	s.e.d.
i. Weight at:			
start (kg)	61.4	63.4	1.1
170 days (kg)	87.7	89.4	1.1
puberty (kg)	94.3	96.5	0.9
second heat (kg)	110.3	114.2	1.1
ii. Period from:			
170 days to puberty (days)	8.6	8.9	1.3
puberty to second heat (days)	21.3	22.6	0.8
iii. Growth rate from:			
170 days to second heat (g/day)	758	800	153
iv. Food conversion ratio from:			
170 days to second heat	4.4	4.0	0.7

Note: No statistically significant difference was observed between treatments for each parameter.

TABLE 34

Results of number of corpora lutea and corpora albicantia for biotin
deficient and supplemented gilts following second oestrus

		Biotin deficient (A)		Biotin supplemented (B)	
		mean	s.e.m.	mean	s.e.m.
Corpora Lutea	Right ovary	7.1	1.1	6.8	0.9
	Left ovary	6.9	0.8	7.3	0.3
	Right and left ovaries	14.0	0.5	14.0	0.5
Corpora Albicantia	Right ovary	4.7	0.7	5.5	1.0
	Left ovary	5.6	0.7	5.3	0.5
	Right and left ovaries	11.1	1.3	10.3	0.5

TABLE 35

Results of ovary weights and reproductive tract lengths for biotin deficient and supplemented gilts following second oestrus

		Biotin deficient (A)		Biotin supplemented (B)	
		mean	s.e.m.	mean	s.e.m.
Ovary	Right	6.9	0.7	7.3	0.6
weight (g)	Left	7.5	0.9	7.7	0.6
Reproductive tract length (mm)	Right	1184	97	1416	141
	Left	1207	116	1455	140

DISCUSSION

The number of ova shed is not usually the limiting factor in restricting litter size, uterine capacity is more important. Uterine capacity is not important up to day 30 of pregnancy but after day 40 it seems that space does limit viable foetuses. Chertkov (1981) showed that litter size was significantly correlated with the length of uterine horn (0.64), ovary weight (0.61) and the number of ovarian follicles of sisters and half-sisters. Cunningham et al. (1979) reported on a trial over nine generations in which they selected for ovulation rate. Both in absolute numbers and in deviation from the control line, there was a significant increase over the generations. Realised heritability was 0.42 ± 0.06 overall, but no significant response in litter size was displayed. Therefore it would appear unlikely that, even if insufficient, dietary biotin would influence litter size through an effect on ovulation rate. A change in uterine capacity, however, could result in a measurable effect. Significantly, no effect on ovulation rate was observed, although a trend towards larger uterine horns resulted from biotin supplementation. The increase in length of the horns was greater than the increase in growth rate of the gilts given biotin supplementation which suggests that the effect on the reproductive tract may be greater than on the overall physiology of the female pig.

However, as this trial was undertaken on a very small number of animals, further investigation of the relationship of dietary biotin intake to uterine capacity and litter performance of the gilt, young and adult sow is indicated.

EXPERIMENT THREE
THE EFFECTS OF DIETARY BIOTIN LEVEL ON THE PHYSICAL PROPERTIES OF THE HOOF HORN AND THE FATTY ACID COMPOSITION OF PERINEPHRIC FAT OF THE PIG

INTRODUCTION

The results obtained in experiment 1 confirmed that the dietary intake of available biotin played an important part in the maintenance of hoof integrity in the pig. However, the mode of action of biotin in this context was not elucidated. It would have been an over-simplification to suggest that certain lesions were characteristic of biotin deficiency. Data accumulated during experiment 1, indicated that differences both in the rate of development and the type of hoof lesion categorised in supplemented and unsupplemented animals, may have resulted from biotin altering the physical characteristics of the horn produced. Such an alteration might affect the horn's durability and ability to withstand traumatic injury.

Two possible modes of action of biotin on the hoof could be proposed. First, the level of dietary biotin might affect the growth rate of horn tissue. If the depression of growth rate which has been reported to occur in biotin-deficient pigs should also occur in hoof horn growth, there might be inadequate compensation for the loss of horn tissue by wear due to abrasive surfaces. This could result in a thinner horn on the volar surface which would be more susceptible to physical damage.

Alternatively, biotin deficiency may alter the durability and resilience of the hoof horn. Geyer (1979) reported that there were less horn tubules per unit area in the biotin-deficient pig. Such a change in horn tubule density might alter the mechanical properties of hoof horn.

The effect of the alteration in the density of horn tubules on the

mechanical properties of the hoof horn might be tested either by examining the compressibility or the penetrability of the horn. If the horn of biotin-deficient pigs could be compressed or punctured more easily, this could help to explain some of the differences which have been observed in previous studies. Punctures are of considerable practical significance as they provide a portal of entry for infections which may result in a breakdown in the integrity of the hoof and subsequently produce lameness.

An important determinant of pigmeat quality is the consistency of carcass fat. Carcasses with soft adipose tissue tend to be unsightly, difficult to cut and are more liable to oxidative rancidity thus reducing the shelf life of the final pork products (Bailey et al., 1973). Fatty acid composition influences the consistency of the fat. Inadequate levels of dietary biotin produce characteristic changes in the fatty acid composition of both liver and adipose tissue. Experimentally-induced biotin deficiency in the pig has resulted in an increase in the unsaturation of adipose tissue (Bühlmann, 1973; L.Volker, 1982, personal communication).

Biotin deficiency produces similar changes in depot fat in avian species (Roland and Edwards, 1971). In addition, it has been shown to produce significant changes in the neutral lipid composition of the foot pad and skin of turkey poults (Logani et al.,1975).

The fatty acid fraction that biotin influences has not been studied in pigs, thus a preliminary fractionation of the depot lipid into phospholipid and neutral lipid would be an important step in assessing the mode of action of biotin on lipid biochemistry in the pig.

The main objective of the experiment reported here was to investigate the relationship between dietary biotin and the durability and resistance of hoof horn. The experimental design also permitted the study of the effect of dietary biotin on fatty acid composition.

The objects of experiment 3 were:-

1)   to develop techniques for measuring the rate of growth and wear of pig hoof horn and to investigate whether a differential rate of

horn growth and wear occurred within different regions of a claw
and between different claws on the same pig;

2) to develop techniques for measuring the durability and resistance
of hoof horn tissue and to investigate the relationship between
these factors and the level of dietary biotin intake;

3) to investigate the effects of the level of dietary biotin intake on
the fatty acid profile of the phospholipid and neutral lipid
fractions of perinephric fat.

MATERIALS AND METHODS

Treatments

Thirty-two gilts were randomly allocated to one of the four following
dietary treatments at 25 kg live-weight:

A. Basal diet plus 5% egg white (negative control);

B. Basal diet;

C. Basal diet supplemented with 120 µg biotin/kg; or

D. Basal diet supplemented with 720 µg biotin/kg.

Two gilts remained on treatment until they reached 86 kg live-weight,
after which weight they were slaughtered. Hoof material and fat
samples were retained at slaughter, the hooves being severed from the
carcass above the coronet prior to scalding.

Diets and Rations

The composition and calculated analyses of the basal diet are given in
Tables 36 and 37. The diets were based on wheat, barley and fishmeal
which are ingredients with a low available biotin content (Table 38).
The basal diet was estimated to supply 22 µg/kg of available dietary
biotin which was equivalent to 2.4 µg biotin/kg metabolic body
weight.

The mineral and vitamin supplements used were the same as those des-
cribed in experiment 1 and the diets were prepared in a similar manner,
giving the dietary levels presented in Table 39.

110

TABLE 36

Percentage composition of experimental diets

	Diets			
	A	B[1]	C	D
Barley	26.3	26.3	26.3	26.3
Wheat	48.5	48.5	48.5	48.5
Wheatfeed	13.2	13.2	13.2	13.2
Fishmeal	10.1	10.1	10.1	10.1
Min/vit supplement	2.0	2.0	2.0	2.0
Dried egg white[2]	5.0	-	-	-
Rovimix H[3]	-	-	0.0012	0.0072

NOTE

[1] Diet B is the basal diet.

[2] Dried egg white contains avidin, a biotin-binding agent.

[3] Rovimix H contained biotin at a 1% inclusion level.

TABLE 37

Calculated analysis of basal diet

Nutrient	Percentage level
Oil	2.1
Protein	16.5
Fibre	3.8
Lysine	0.84
Methione and Cystine	0.58
Threonine	0.56
Calcium	1.20
Phosphorus	0.78
Salt	0.53
DE	13.2 MJ/kg

TABLE 38

Calculated available biotin content of basal diet

	% Diet	Assumed biotin level (μg/kg)	Total biotin (μg/kg)	Assumed Availability	Available biotin (μg/kg)
Barley	26.26	150	39	30	11.7
Wheat	48.45	100	48	10	4.8
Wheatfeed	13.19	310	40	10	4.0
Fishmeal	10.10	110	11	30	3.3
Min/vit	2.00	–	–	–	–
Total	100.00		138		23.8

TABLE 39

Vitamin and mineral supplements

Vitamin A	15000 iu/kg
D_3	2000 iu/kg
E	20 iu/kg
K	4 mg/kg
B_1	2 mg/kg
B_2	5 mg/kg
B_6	4 mg/kg
B_{12}	15 µg/kg
Nicotinic acid	18 mg/kg
Pantothenic acid	15 mg/kg
Folic acid	1 mg/kg
Choline	200 mg/kg
Iron	100 g
Cobalt	1.5 g
Manganese	50 g
Copper	10 g
Zinc	80 g
Iodine	3 g
Selenium	0.1 g
plus calcium, phosphorus and salt	

Diet B was the basal diet. Diet A contained, in addition, 5% egg white as a source of avidin, a biotin-binding agent at a level calculated to be more than sufficient to bind all the biotin present in the raw materials (Appendix 12).

Diets C and D were produced by supplementing the basal diet with 120 µg/kg and 720 µg/kg of biotin respectively in the form of Rovimix H (Roche Products Ltd).

During the pre-trial period the gilts were fed ad lib. a proprietary cubed rearing diet. At 25 kg live-weight they were transferred to one of the four treatments and were individually fed once daily, in the morning, to a scale which allowed 110 g/kg metabolic body weight (Table 40). Gilts on treatment A were fed a 5% higher scale to ensure that the intake of all other nutrients was at least equal to that on the basal diet.

The level of biotin added in treatment C was equivalent to 13 µg/kg metabolic body weight in the diet as fed (plus a small natural contribution). The level in treatment D was six times greater (79 µg/kg metabolic body weight) which is equivalent to that suggested as a restorative level for sows following breakdown of hoof integrity due to dietary biotin deficiency (Glättli, 1975).

Housing
The gilts were housed in rearing accommodation divided into 4 pens, each holding 2 gilts from each treatment, for the duration of the trial. The flooring was solid concrete with a lightly strawed sleeping area and a separate dungingpassage. Individual feeding stalls were provided.

Plasma Biotin Level
Blood samples were taken from the trial pigs by jugular puncture prior to 43 days and at 84 days or immediately prior to slaughter should that have been sooner. The samples were prepared and assayed as described in experiment 1.

Growth Rate and Food Conversion Ratio
The feed consumed each day for the duration of the trial was recorded and the gilts were also weighed on each Monday so that growth rates and

115

TABLE 40

Daily rations of experimental animals' feed levels related to metabolic body weights

Liveweight kg	Metabolic Body wt ($W^{0.75}$) kg	Treatments B, C and D ration kg/pig/day	Treatment A ration kg/pig/day
25	11.2	1.23	1.29
30	12.8	1.41	1.48
35	14.4	1.58	1.66
40	15.9	1.75	1.84
45	17.4	1.91	2.01
50	18.8	2.07	2.17
55	20.2	2.22	2.33
60	21.6	2.38	2.50
65	22.9	2.52	2.65
70	24.2	2.66	2.79
75	25.5	2.81	2.95
80	26.7	2.94	3.09
85	28.0	3.08	3.23

NOTE: 1) Estimated feed intake per kg metabolic body weight = 0.11 kg.

2) Treatment B ration scale at 5% higher level.

food conversion ratios could be calculated and feed allocations adjusted.

Rate of Growth and Wear of the Hoof Horn

The technique used to investigate the rate of growth and wear of pig hoof horn was based on a method used by Murphy (1978) to study the growth rate of hoof horn of beef cattle. A study was undertaken to examine whether this technique could be applied to pigs and whether a differential rate in horn growth and wear occurred within different regions of a olaw.

A permanent mark was imparted at three distinct regions of the outer side-wall of the hoof horn (Figure 25). The rate of growth of horn tissue could be estimated by measuring the distance from the coronet to the mark and calculating distances with time. The rate of wear could be estimated from the mark to the volar edge, which was in contact with the floor. Compartmentalising the side-wall into regions permitted an analysis of any differential rate of growth and wear of these regions to be undertaken. Murphy (1978) suggested that, in growing cattle, such a differential rate in horn growth and wear occurred due to change in distribution of an animal's mass.

The study was undertaken on the inner and outer claws of the right-hand feet of the animals fed the basal diet. The pigs were initially captured manually; the older animals were nose-tethered. A specially designed instrument, when heated, was used to brand hoof horn, imparting a small cross-shaped mark to the horn at about 5 mm from the coronet. Three distinct positions were chosen for branding (Figure 25). Position 1 was 5 mm posterior to the leading edge of the side-wall; position 3 was 10 mm anterior to the posterior edge and position 2 was equidistant between positions 1 and 2.

The measurements of growth (the distance from the junction of the coronet/skin to each mark) and wear (the distance from the mark to the volar edge of the horn) were made at 21 day intervals.

Physical Tests on Hooves

i) Compression Test

The compression test was conducted in order to provide an objective

FIGURE 25

Growth and wear test

Sampling areas denoted
by x-shaped marks
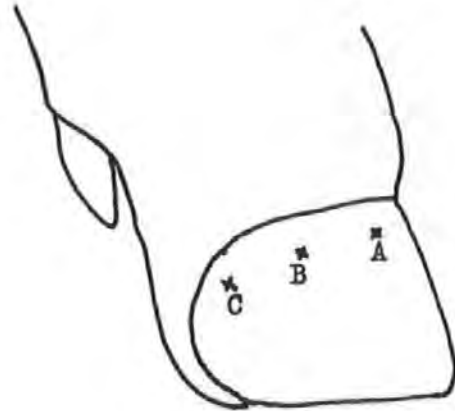
FIGURE 26

Compression test

Sampling region denoted
by circle
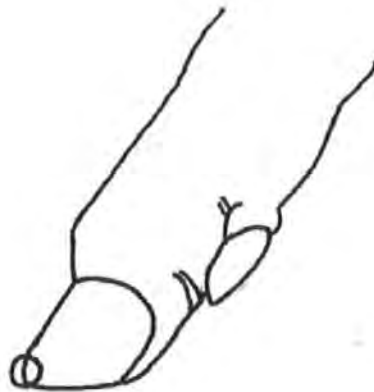
FIGURE 27

Puncture test

Sampling regions
denoted by circles

Area B   Area A

118
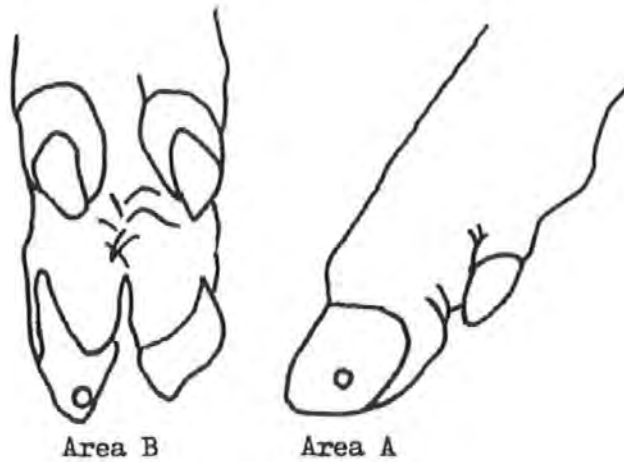
measure of the compression yield strength of hoof horn. The testing of the samples was conducted on an Instron Materials Testing Instrument. A load cell of 50 kg maximum capacity was employed. The crosshead speed was 100 mm per minute and the chart speed was 200 mm per minute. For a compression test to be undertaken, horn samples had to be of adequate dimensions which could only be achieved from the leading edge of the side-wall of an outer claw in pigs of 86 kg liveweight (Figure 26). Rectangular samples (3.2 mm by 1.6 mm by 3.2 mm) were taken from the front outer claw. In most cases only a single sample could be obtained due to the small size of the hoof. The inability to take more than one sample limited assessment of repeatability of the results.

The samples were compressed in a plane perpendicular to the orientation of the horn tubules until the samples sheared. This was the yield point. A trace was produced on a chart from which values for compression yield strength and tangent modulus of elasticity were calculated.

ii) Puncture Test

This test was chosen as it was thought it might mimic the effect of sharp stones or other projections which may penetrate the hoof. Samples of horn from two predetermined areas were dissected from under-lying tissue (Figure 27). Area A was on the abaxial side-wall of the outer claw, at a point halfway from the leading edge to the rear edge on the horizontal axis and two-thirds of the distance from the coronet to the volar edge on the vertical axis. Area B was on the volar surface of the outer claw immediately behind the white line at the leading edge of the toe.

These regions were chosen as they were flat areas and consequently enabled the samples to be clamped firmly with the surface perpendicular to the test probe. Using the Instron Materials Testing Instrument prepared as previously described, the samples were pierced perpendicularly using a 3 mm probe. A measure of the load necessary for complete piercing of the horn was obtained from the chart.

iii) The Durometer

Both the previous tests involved a considerable amount of sample preparation and were, by necessity, performed on morbid material. Preliminary investigations were also conducted using a small hand-held

119

device called a Durometer (Shore Manufacturing Company) to see if non-destructive measurements of hoof hardness could be made on the live animal. The Durometer was developed for the non-destructive testing of rubber and rubber-like materials. In principle, the Durometer should not cause damage to the hoof or cause pain when used on the live animal. It operates by pressing a small probe against the material to be tested from which a direct measurement is obtained.

Measurements were made on the outer side-wall of the whole outer claw from the left-hand side of the pig. A series of measurements were taken on the side-wall along a line bisecting the coronet and volar edge (Figure 28). Heel measurements were taken along a line bisecting the ball of the heel laterally from the axial to abaxial side. A measurement was taken from the anterior toe region and two measurements from the anterior white line region.

Fatty Acid Assay

Preliminary fatty acid analyses and iodine values were performed on perinephric fat samples to ascertain whether biotin deficiency was being achieved on Diet A as calculated.

i) Iodine value

Iodine values were taken to measure the degree of unsaturation of fat samples.

About 0.25 g of fat sample was accurately weighed into a clean conical flask. The fat was dissolved with 10 ml of carbon tetrachloride and 25 ml of Wij's solution added. The flask was stoppered, mixed and allowed to stand in the dark for thirty minutes. To the flask was then added 20 ml of 10% potassium iodide solution to liberate the iodine from the excess Wij's solution, and 100 ml of distilled water. The whole was then titrated against sodium thiosulphate. When a pale yellow colour was achieved, 2-3 ml of starch solution was added so that the titration end-point could be clearly perceived as a colourless solution. The amount of liberated iodine could be ascertained from the titration.

A blank titration was carried out at the same time in which the thiosulphate was titrated against the preparation desribed above, but

FIGURE 28

Durometer sample regions

Test areas

Heel

Toe
White line
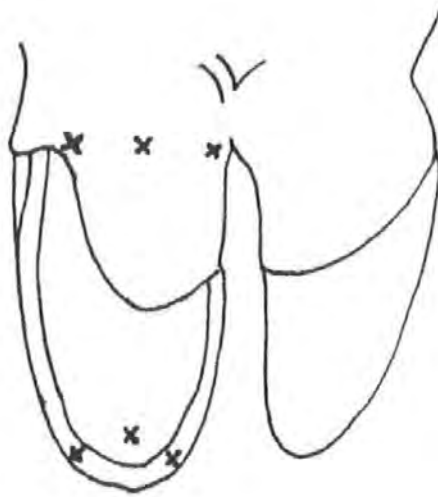
Volar view

Sidewall view
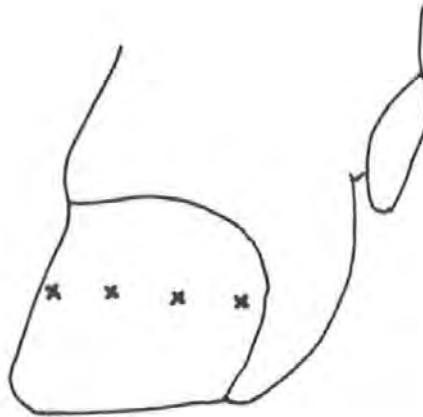
Sidewall

without the fat sample. The difference between the volume of thio-
sulphate used in the blank and in test samples gave the amount of
thiosulphate equivalent to the iodine absorbed by the fat.

ii) Fatty acid extraction of perinephric fats
Fat samples were prepared for assay on a gas liquid chromatograph (GLC).
About 0.5 g sample of fat was freeze-dried to remove excess moisture
prior to methylation.

The samples were methylated using the following procedures: 5 ml of
methanol   and 0.5 g of sodium hydroxide were added to the fat
sample and refluxed for ten minutes in a Quickfit reflux apparatus.
5 ml of boron⁺⁻ifluoride/methanol complex were added to the mixture and
the whole refluxed for two minutes. This produced the methyl esters to
which 5 ml of n-heptane were added and the whole refluxed for a final
two minutes. The reflux container was filled with saturated salt
solution to enable the upper heptane layer, which contained the esters
to be pipetted off into a clean specimen tube. Sodium sulphate was
then added to dry the solution. (Horowitz 1970).

The solution containing the methyl esters was analysed by gas liquid
chromatography. The GLC was a Pye 104; the liquid phase was diethy-
lene glycol succinate; and the temperature was set at $178.5^{\circ}C$. The
details for the GLC were unchanged for all subsequent analyses. The
gas liquid chromatography allowed the relationship between palmitic
acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0) and oleic
acid (C18:1) to be examined.

This shortened technique of the more complete assay used for production
of fatty acids from phospholipids and neutral lipids, allowed for a
rapid assessment of the fatty acid profile of the experimental pigs.

iii) Extraction of fatty acids from neutral lipids and phospholipids of
perinephric fat

About 0.5 g of fat was freeze-dried and then refluxed for twenty-four
hours, to extract the fats, with about 100 ml of chloroform:methanol
(2:1 mixture) in a soxhlet extraction. The lipid extract was trans-
ferred to a separating funnel and washed with two times equal volumes
of water. The lipid was recovered by removal of the bulk of the

solvent by distillation; the residual solvent was evaporated under a jet of nitrogen.

The lipid was dissolved in a minimum volume of chloroform and twenty volumes of acetone was added. The mixture was left at 0°C overnight for the phospholipids, which are not soluble in acetone, to precipitate. The phospholipids were removed by centrifugation and the neutral lipid fraction recovered by evaporation of the solvent as before. From this point the two samples were treated in the same way.

The lipid material was dissolved in 20 ml of ethanol and 2 ml of 60% aqueous potassium hydroxide was added. The mixture was refluxed for one hour and then transferred to a separating funnel with two volumes of water. The mixture was extracted three times with 10 ml portions of petroleum ether to remove the non-saponifiable lipids. The aqueous solution remaining (which contained the fatty acids as their potassium salts) was then acidifed with dilute hydrochloric acid producing free fatty acids which were recovered by extracting twice with 10 ml portions of petroleum ether. The fatty acids were recovered by evaporation of the solvent under a jet of nitrogen.

To the dry fatty acid material, 4 ml of boron trifluoride/methanol complex was added, the mixture boiled for two minutes and then poured into 15 ml of distilled water in a separating funnel. This procedure generated the fatty acid methyl esters which were extracted into 5 ml of petroleum ether and recovered by evaporating the solvent under a jet of nitrogen.

Some samples were contaminated with non-polar material which interfered with the GLC. This was removed by Thin Layer Chromatography (TLC) on silica gel with benzene:hexane (40:60 mixture) as developing solvent. The fatty acid methyl esters ran together as a band with an Rf of about 0.3 and were identified by their correspondence with a reference marker of any fatty acid methyl ester (stearic acid was used). The material was visualised by spraying the plate with a dilute solution of Rhodamine-6G or dichlorofluoroscein in acetone (adsorption indicators). The esters were recovered by scraping off the portion of the silica gel containing them. A thin layer of chromato-graphic alumina was then placed in a scintered glass funnel and the

silica gel placed on top of this. This was washed through with a small amount of ether, the esters were eluted and the adsorption indicator retained by the alumina.

RESULTS

All gilts completed the trial.

Plasma Biotin Levels

The technique of jugular puncture resulted in a high proportion of samples being unacceptable for the sensitive assay technique. Two main reasons for failure were that sufficient blood could not be obtained from some gilts and red blood cells haemolysed preventing separation of plasma in other samples.

Increased dietary biotin intake resulted in higher plasma biotin levels (Table 41). The treatment differences were constant with age.

Growth Rate and Food Conversion Ratio

Neither daily live-weight gain (DLWG) nor food conversion ratio were significantly influenced by treatment (Table 41), although DLWG tended to be higher in treatment A.

Rate of Growth and Wear of the Hoof Horn

Measurements were not obtained from all periods of the investigation. This was due to the marks which were imparted on the hoof either growing out from, or being worn off the horn. Position 3 failed to provide complete data by the second reading. The final period provided insufficient readings from any position for statistical analysis.

No statistically significant difference was recorded for the first and second periods of growth, between either: 1) the positions on each claw; 2) the same position on the outer and inner claws; and 3) the same position on the front and hind claws (Table 42). The rate of growth appeared to decline between the first and the second three week periods.

The same parameters were also analysed statistically for wear. Statistical significance was found only between the positions on the inner claws on front and hind feet respectively following the first three weeks of study. The rate of wear was significantly faster in

TABLE 41

Effect of dietary treatment on plasma biotin level and performance characteristics of growing pigs

	Treatment				s.e.d.	Level of significance of treatment differences	One-way[1] analysis of variance (5% level)
	A	B	C	D			
Daily liveweight gain (g/day)	764	720	696	706	26	N.S.	
Food conversion ratio	2.83	2.81	2.88	2.86	0.12	N.S.	
Plasma biotin level < 43 days	37	46	87	439	50	***	ABCD
(ng/100 ml) > 43 days	36	71	107	474	52	***	ABCD

NOTE

[1]Means joined by line are not significantly different.

TABLE 42

Assessment of rate of growth and wear of side-wall regions of fore and hind claws

Parameter	Period	Fore Outer Position 1		2		3		Fore Inner Position 1		2		3		Hind Outer Position 1		2		3		Hind Inner Position 1		2		3	
		mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.
Growth (mm)	1	0.26	0.04	0.32	0.02	0.35	0.03	0.33	0.02	0.32	0.02	0.37	0.02	0.36	0.03	0.35	0.03	0.34	0.03	0.36	0.03	0.32	0.03	0.33	0.03
	2	0.36	0.06	0.30	0.06	0.26	0.07	0.24	0.04	0.27	0.05	0.32	0.08	0.28	0.05	0.31	0.08	0.30[2]		0.32	0.06	0.27	0.05	0.33[2]	
	3	0.52[2]		0.44[2]		0.58[2]		0.43	0.01	0.44[2]		[1]		0.56[2]		0.54[2]		0.58[2]		0.52[2]		0.52[2]		0.46[2]	
Wear (mm)	1	0.22	0.01	0.18	0.03	0.22	0.05	0.18	0.02[a]	0.21	0.03[a]	0.30	0.03[a]	0.20	0.02	0.20	0.40	0.17	0.04	0.25	0.02[b]	0.24	0.01[b]	0.18	0.02[b]
	2	0.15	0.06	0.16	0.07	0.21	0.09	0.16	0.03	0.18	0.05	0.20	0.11	0.19	0.03	0.20	0.11	0.30[2]		0.21	0.04	0.12	0.03	0.43[2]	
	3	0.29[2]		0.31[2]		0.29[2]		0.38	0.02	0.35[2]		[1]		0.44[2]		0.50[2]		0.21[2]		0.31[2]		0.31[2]		0.25[2]	

Note: [a] Figures with superscript a are statistically significantly different from each other (p < 0.01)

[b] Figures with superscript b are statistically significantly different from each other (p < 0.05)

[1] No pigs provided data

[2] Only one or two pigs provided data; no analysis undertaken

position 3 on the front inner claw compared with positions 1 and 2.
Conversely, the rate of wear was significantly slower in position 3 on
the hind inner claw compared with positions 1 and 2. The results from
the third period indicated that the rate of wear was greater compared
to the previous periods.

Generally the rate of growth exceeded the rate of wear.

Physical Tests on Hoof Horn

i) Compression Test

No significant treatment differences were displayed for compression
yield strength or modulus of elasticity (Table 43). However compres-
sion strength did increase with dietary biotin intake.

ii) Puncture Test

No significant variation between treatments was observed for either toe
or side-wall measurements (Table 43).

iii) The Durometer

The Durometer measurements varied between regions (Table 44). The
lowest readings were from the heel region. The white line region
produced readings which were lower than the median toe region or the
side-wall. Treatment A produced a lower value for the side-well, toe
and white line regions, the other treatments produced similar results.

Fatty Acid Analysis

i) Iodine Values

The iodine values were 72.9, 48.9, 51.5 and 51.6 (s.e.d. $\pm$ 3.0) for
treatments A, B, C and D respectively. This result shows the fats from
treatment A were more unsaturated ($p < 0.001$) than the other treatments.
Treatments B, C and D exhibited little variation.

ii) Total Fat Analysis

The level of unsaturation of total fats was greatest ($p < 0.001$) in
treatment A compared to the other treatments (Table 45). The results
for the other treatments were similar. Each fatty acid responded to
the change in saturation by significant increases in the relative
proportions of the individual and combined monounsaturates ($C16:1$ and
$C18:1$) and similarly, in decreases of the saturates ($C16:0$ and $C18:0$).

TABLE 43

Effect of dietary biotin intake on physical characteristics of hoof horn

| | Treatment | | | | s.e.d. |
	A	B	C	D	
Puncture test					
Side-wall (N)	327	335	325	317	25
Toe (N)	326	408	337	374	31
Compression yie_ strength (MNm^{-2})	27.8	28.9	30.3	35.5	4.4
Modulus of elasticity (MN^{-2})	297	426	280	382	84

128

TABLE 44

Horn hardness measurements using a Durometer

	Treatments				s.e.d.
	A	B	C	D	
Side-wall	56.5	59.4	59.4	58.5	1.6
Heel	8.9	4.6	6.4	8.6	1.2
Toe	39.0	43.8	43.0	41.8	2.9
White line	32.8	34.8	33.0	36.3	1.7

129

TABLE 45

The effect of dietary biotin intake on the percentage fatty
acid composition of perinephric fat for total fat analysis

Fatty acid	Treatment				s.e.d.	Level of significance of treatment effects	One-way analysis of variance (5% level)
	A	B	C	D			
C16:0	26.2	33.0	32.8	33.3	1.1	***	ABCD
C16:1	8.2	3.5	2.3	2.8	0.7	***	ABCD
C18:0	13.0	23.9	24.1	25.0	1.3	***	ABCD
C18:1	53.0	40.3	40.9	39.8	1.3	***	ABCD
C16:0 + C18:0	38.2	56.2	56.9	57.6	1.8	***	ABCD
C16:1 + C18:1	61.2	43.8	43.1	42.6	1.9	***	ABCD
Monoene:saturated ratio	1.6	0.78	0.76	0.74			

This was clearly illustrated by the monoene:saturated ratio.

iii) Fractionated Analysis

The phospholipid and neutral lipid fatty acid fractions were influenced by dietary biotin intake (Table 46). The relative percentage of the saturated fatty acids, C16:0 and C18:0, compared to the monounsaturates, C16:1 and C18:1, significantly increased with dietary biotin intake except at the highest levels of intake (treatment D). The fatty acids of the gilts fed treatment D exhibited a slightly higher level of unsaturation compared to those fed treatment C, the medium dietary biotin formulation. Each of the individual fatty acids showed the same trend, significant, in the case of C18:0 and C18:1, in both lipid fractions. The neutral lipid fraction was more unsaturated than the phospholipid fraction. However the only statistically significant difference occurred in the case of C18:1 which differed significantly between all treatments (p <0.05 or greater).

DISCUSSION

Tagwerker (1973) stated that groups of biotin-deficient piglets had plasma biotin concentrations of 20 to 30 ng/100 ml compared with 40 ng/100 ml in normal piglets. The pigs given treatment A, with the egg-white supplement diet, had plasma biotin levels within the range assumed to be indicative of a biotin deficiency when sampled in both periods of the trial. Clinical symptoms of biotin deficiency were not observed. In fact, the growth rate of the biotin-deficient (treatment A) gilts tended to be greater than the other treatments. This may be explained by the higher total energy and protein provided in the treatment as a result of the addition of 5% dried egg white.

The technique for determining the rate of wear and growth of the hoof horn provided variable results. In particular it was discovered that a twenty-one day period between measurements permitted too many values to be lost, particularly from the third position of the horn. The data reported here produced growth rate estimates which ranged from 0.250 to 0.375, 0.250 to 0.375 and 0.429 to 0.571 mm/day for periods 1, 2 and 3 respectively (excluding data from position 3). There were few results from the third period, but the results from periods 1 and 2 compared well with those reported by Geyer (1979). He stated that the rate of

131

TABLE 46

The effect of dietary biotin intake on the percentage fatty acid composition of
the neutral lipid and phospholipid fractions of perinephric fat

Fatty acids	Treatments				s.e.d.	Level of significance of treatment effects	One-way analysis of variance (5% level)[1]
	A	B	C	D			
Phospholipids							
C16:0	28.4	30.7	34.1	33.4	3.4	N.S.	
C16:1	8.0	13.4	10.7	8.3	3.0	N.S.	
C18:0	14.2	14.3	21.5	19.1	2.6	*	ABDC
C18:1	49.6	41.7	33.8	39.3	2.9	***	ABCD
C16:0 + C18:0	42.6	44.9	55.6	52.5	3.5	**	ABCD
C16:1 + C18:1	57.5	55.1	44.5	47.9	3.6	**	ABDC
Monoene:saturated ratio	1.34	1.23	0.80	0.91			
Neutral Lipids							
C16:0	23.8	27.6	29.9	26.8	2.4	N.S.	
C16:1	10.4	12.4	10.3	11.5	2.5	N.S.	
C18:0	9.3	10.5	15.9	13.6	2.1	*	ABDC
C18:1	56.6	49.6	44.0	48.1	3.4	*	ABCD
C16:0 + C18:0	33.2	38.1	45.7	40.4	3.5	***	ABDC
C16:1 + C18:1	66.8	62.0	54.4	59.5	3.5	***	ABDC
Monoene:saturated ratio	2.02	1.63	1.19	1.47			
Level of significance of difference between fractions							
C16:0	N.S.	N.S.	N.S.	**			
C16:1	N.S.	N.S.	N.S.	N.S.			
C18:0	**	N.S.	N.S.	N.S.			
C18:1	**	*	*	*			
C16:0 + C18:0	**	N.S.	*	**			
C16:1 + C18:1	**	N.S.	*	**			

[1]Means joined by a line are not significantly different.

growth for a pig up to three months of age was 10 mm/28 days or 0.375 mm/day. The rate of wear was less than the rate of growth, which not unexpectedly, showed that the hooves were increasing in size. The interval between measurements should have been shorter but the measurements did permit an assessment of the relationship between growth and wear of hoof horn. Thus the effect of dietary biotin intake on this relationship could be examined.

The effects of dietary biotin intake on the physical properties of hoof horn were investigated using the Instron Materials Testing Instrument. Only compression yield strength tended to increase with biotin supplementation. Although the technique looked promising, it was not possible to obtain sufficient and large enough samples from a claw of the young gilt of 80 kg live-weight. Only a single very small sample could be obtained from the larger claw of the foot examined for the compression yield strength and modulus of elasticity test. Few problems were encountered when using the Durometer. The biotin-deficient (treatment A) gilts gave lower readings, for all areas except the heel. This showed that these gilts had a less firm hoof horn which agreed with previous descriptions of soft hoof horn in the biotin-deficient pig (Tagwerker, 1973; Whitehead et al., 1980). The values for the soft heel were very low and a confounding effect may have arisen from the pressure exerted by the operator. It also became clear that readings taken near a crack were unreliable and consequently such readings were excluded from the data.

The results of the fatty acid analyses also confirmed that the treatment A group were biotin-deficient. The analyses on unfractionated fat for iodine values and by gas-liquid chromatography showed treatment A to have more unsaturated fats than the other treatments. Hard, medium hard, medium soft, soft and oily pig fats have been given iodine values of 63, 68, 71, 77.5 and 88 respectively (Hankins and Ellis, 1926). Lea et al.(1970) quoted R.G.Baskett (1938, unpublished memorandum) as defining firm, medium and soft fats in bacon pigs as having iodine values from 65, 65-70 and 70 respectively. The mean iodine value for the perinephric fat of the treatment A gilts was 72.9 and therefore would be classified as a soft fat, which is indicative of biotin deficiency (Bühlman, 1973). The gilts on the other treatments recorded values similar to those given by Lea et al.(1970) for the perinephric

fat of Large White pigs slaughtered at about 90 kg live-weight. The iodine values and gas-liquid chromatography of the unfractionated fats failed to display a treatment difference between the biotin-unsupplemented (B) and the biotin-supplemented treatments (C and D). As the mean plasma biotin levels for treatment B were above the levels considered to indicate deficiency, this may be expected. However, the fractionated analysis of the perinephric fat showed a general trend of increasing fatty acid saturation with increasing dietary biotin intake, although at the highest level of biotin supplementation, a slight decrease of the saturation of the fatty acids was observed. Usually vitamins can be absorbed in large quantities without ill-effects. This may have been the first symptoms of hypervitaminosis but this condition has not been observed previously with biotin. Further work would have to be undertaken to assess whether it was a real effect.

The results of the fractionated analyses are discussed in more detail together with the results of experiment 4.

EXPERIMENT FOUR
THE EFFECT OF A CONSTANT AND CHANGE OF DIETARY BIOTIN INTAKE ON THE FATTY ACID COMPOSITION OF PERINEPHRIC AND HOOF HORN FAT AND THE PHYSICAL PROPERTIES OF THE HORN

INTRODUCTION

The results from experiment 3 gave some indication of the character of the biotin-deficient hoof horn. Compressive yield strength of the hoof horn of a pig on a biotin-deficient diet tended to be lower. The young female pig on a diet of low biotin content developed more lesions as experiment 1 progressed, but the first assessment at 170 days of age showed similar results for both treatments. Therefore the development of lesions in the young gilt and their relationship to the durability of the hoof are not clear. Unexpectedly the gilts on experiment 3 showed no treatment effect on either DLWG or FCR despite other measured responses to dietary biotin intake. Growth rate of the hoof horn may not be influenced by dietary biotin intake, but it might be expected that the rate of wear of the weaker horn of a pig on a biotin-deficient diet would be greater. Alternatively, the biotin status of a pig may influence the horn tissue itself, altering its resistance to traumatic injury. Tagwerker (1973) and Whitehead et al. (1980) both observed that horn tissue was rubbery and softer in a biotin-deficient pig. Some subjective observations in field cases (Comben, 1978) also suggested that sows on suspectedly biotin-deficient diets had softer hoof horn. Changes in the fatty acid composition of the horn, mediated by the biotin regime, may have resulted in these effects. If the increase in unsaturation of fatty acids that occurred in the perinephric fat accompanying dietary biotin deficiency (shown in experiment 3) also occurred in the hoof horn, an increase in the permeability of the horn to water may have resulted.

The effects on hoof horn may also be influenced by short-term changes

in dietary biotin intake. On commercial units, sows previously fed
suspectedly biotin-deficient diets were given very high levels of
dietary biotin which produced a rapid recovery in hoof health. When
clinically healthy, the sows were placed on lower biotin intake
estimated to be adequate to meet the normal nutritional requirements.
Subjective assessments of their health subsequent to this change in
biotin level suggested that the sows suffered a regression to a biotin-
deficient state. This appeared to be a temporary phenomenom while the
pig adjusted to the new dietary regime (Comben, 1978).

The design of the experiment reported here permitted the investigation
of whether this transient state occurred and further studied the fatty
acid profile and durability of hoof horn tissue. The rate of growth
and wear and the state of the hooves were also examined.

The object of experiment 4 was to investigate the effect of dietary
biotin intake on:

1) the rate of growth and wear of the hoof horn;
2) the number of defects and lesions in the hoof horn of young gilts;
3) the durability of hoof horn tissue measured using a Durometer;
4) the fatty acid profile of the phospholipid and neutral lipid
fractions of perinephric and extractable hoof horn fat.

The trial also investigated whether short-term biotin deficiency
occurred on reduction of biotin intake and its effect on the above
parameters.

MATERIALS AND METHODS

Treatments

Four replicates, each of eight gilts, were fed either:

A. Basal diet plus 5% egg white;
B. Basal diet;
D. Basal diet plus 720 µg/kg supplementary biotin; or
E. Basal diet plus 720 µg/kg supplementary biotin for six weeks,
    changing to the dietary regime of treatment B for the remainder
    of the trial.

The gilts were randomly allocated to one of the four treatments at 20 kg

live-weight and remained on treatment until they reached 80 kg live-weight, after which weight they were slaughtered. Hoof material and fat samples were retained at slaughter, the hooves being severed from the carcass above the coronet prior to scalding.

Diets and Rations

The dietary formulations were described in experiment 3. Diets for treatments A and B were as in experiment 3 (page 110) treatments D and E were initially fed a diet supplemented with 720 µg biotin/kg which provided a daily intake equivalent to the restorative level for a sow following a dietary regime suspectedly deficient in biotin suggested by Glättli (1975). After six weeks the gilts on treatment E were fed the basal diet (treatment B) for the remainder of the trial.

During the pre-trial period the gilts were fed a proprietary cubed rearing diet ad lib. At 20 kg live-weight they were transferred to one of the four treatments and were individually fed once daily, in the morning, to the scale in Table 47, which allowed 93 g/kg metabolic body weight. The level of feed allowed per day was slightly lower than in experiment 3 as there was evidence that gilts, especially those on treatment A fed at a 5% higher scale, were not able to accept all the offered feed. Thus in experiment 4 the basal treatment supplied 2.1 µg available biotin/kg metabolic body weight. The level of biotin in treatments D throughout the trial and E for the first six weeks was equivalent to 67 µg/kg metabolic body weight (plus a small natural contribution).

Plasma Biotin Level

Blood samples were taken by jugular puncture. Samples were taken from all gilts prior to 43 days and at 12 weeks or prior to slaughter should that have been sooner. In addition samples were taken from four gilts each on treatments B and D at seven weeks. The samples were prepared and assayed as described in experiment 1.

Growth Rate and Food Conversion Ratio

The feed consumed each day for the duration of the trial was recorded and the gilts were weighed on Monday of each week in order that growth rates and food conversion ratios could be calculated and feed allocations adjusted.

TABLE 47

Daily rations of experimental animals' feed levels related to metabolic body weight

Liveweight (kg)	Metabolic body weight (kg)	Treatments B, D and E ration (kg/pig/day)	Treatment A ration (kg/pig/day)
20	9.5	0.88	0.92
25	11.2	1.05	1.10
30	12.8	1.19	1.25
35	14.4	1.35	1.42
40	15.9	1.48	1.55
45	17.4	1.63	1.72
50	18.8	1.75	1.84
55	20.2	1.89	1.98
60	21.6	2.00	2.10
65	22.9	2.14	2.25
70	24.2	2.25	2.36
75	25.5	2.39	2.51
80	26.7	2.48	2.60

Hoof Records

After the gilts had been slaughtered the feet were removed and frozen and subsequently individually examined. Detailed records were made of location and type of defect and lesion present in the hoof. The injuries were categorised as described in experiment 1.

Rate of Growth and Wear of the Hoof Horn

The same technique for imparting permanent marks on, and measuring the rate of growth and wear of, hoof horn was used as described in the previous experiment. In the experiments a single mark was imparted on the outer claws of the fore and hind feet from the left side of all gilts. This enabled treatment differences to be studied in the rate of growth and wear. In order to assess the effects of dietary change at 6 weeks, two experiments were undertaken in which measurements were taken over two consecutive periods of time: before the dietary change (0-6 weeks) in the first experiment; and after the dietary change (7 weeks to slaughter) in the second experiment. The interval between measurements was reduced to 14 days because it had been found that marks grew out during the 21 day interval in the previous experiment. The rate of growth and wear of each 14 day period was assessed.

Durometer Measurements

The mode of action of the Durometer was explained in experiment 3. Further studies were carried out on all claws of the gilts. The claws were first defrosted and a series of readings were taken on the side-wall along a line bisecting the coronet and volar edge. At the leading edge two further readings were taken 5 mm dorsal and ventral to the centre measurement. Two measurements were taken on the median region of the toe 5 mm apart.

Fatty Acid Extraction from Neutral Lipids and Phospholipids

Fatty acid extractions were performed on:

i) perinephric fat; and
ii) pig hoof horn.

The technique for perinephric fat was described in experiment 3. Modifications in method for pig hoof horn were as follows. The hoof horn was excised from underlying tissue and prepared for passage through

a bench mill. This resulted in fine flakes upon which the extraction procedure could be performed.

The flakes were extracted with about 100 ml of chloroform:methanol (2:1 mixture) in a soxhlet extraction for two days. The methanol freed those phospholipids bound in protein. Subsequent procedure was as described for perinephric fat.

<center>RESULTS</center>

Animals Providing Data

A single gilt from treatment B died during the trial, all others completed the trial. After a month on trial, the gilts suffered from scouring due to <u>vibrio</u> sp. virus. All recovered after treatment with antibiotics but for the period of the illness there was little intake of solids.

Biotin Assays

The technique of jugular puncture resulted in a high proportion of samples being unacceptable for the sensitive assay technique. Two main reasons for failure were that sufficient blood could not be obtained from some gilts and red blood cells haemolysed preventing separation of plasma in other samples.

Increased dietary biotin intake resulted in higher plasma biotin levels (Table 48). These levels increased in the older pigs. The levels for treatment E were similar to treatment D for pigs fed the same diet for the first six weeks on trial. For both treatments, the plasma biotin levels were significantly higher than treatments A and B. Following the change to the treatment B diet, the plasma biotin levels of the treatment E pigs were reduced to the same level as those gilts maintained on the treatment B diet. Treatment A tended to show the lowest level of plasma biotin.

Growth Rate and Food Conversion Ratio

Daily live-weight gain was significantly higher ($p < 0.01$) and food conversion ratio lower for the animals in treatment A (Table 48).

Hoof Studies

The highest number of claws affected by defects was shown for treatments

<center>140</center>

TABLE 48

Effect of treatment on plasma biotin level and performance

	Treatment				s.e.d.	Level of significance of treatment effects	One-way analysis of variance[1]
	A	B	D	E			
Daily live-weight gain (g/day)	636	568	578	562	19	**	<u>AB</u>DE
Food conversion ratio	2.68	2.73	2.77	2.72	0.05	NS	
Plasma biotin level ◁ 43 days	28	69	744	718	277	***	<u>AB</u><u>DE</u>
(ng/100 ml) ▷ 43 days	168	221	1019	275	89	***	<u>DA</u><u>BE</u>

[1]Means joined by a line are not significantly different.

B and D (3.7 $\pm$ 0.7 and 3.1 $\pm$ 0.7 , respectively) compared to 1.9 $\pm$ 0.4 and 1.8 $\pm$ 0.6 for treatments A and E (Table 49). A similar trend was displayed for the number of defects/pig with 4.57 and 3.88 for treatments B and D compared to 2.63 and 2.38 for treatments A and E (Table 50). Treatment D excepted, the fore inner claws exhibited more injury through defects than the fore outer claws. However, the outer claws of the hind feet exhibited the greater degree of damage through defects than the inner hind claws. Most of the injury was caused by bruising and abrasion, particularly of the toe region, although other regions suffered equal damage in treatment B (Table 51). Treatment B also showed the greatest proportion of cuts in the soft heel. Claws were affected by lesions to a greater degree than by defects. Treatment A showed 4.1 $\pm$ 0.3)claws affected by lesions which was significantly less (p <0.05) than the other treatments (Table 52). Treatments B, D and E showed a similar level of over 5.0 claws with lesions. However, treatment E showed the least number of lesions/claw at 15.9 and treatment D the highest at 20.5 (Table 53). The outer claws of the fore and hind feet exhibited a greater degree of damage due to lesions compared to the inner claws for all treatments. The lesions were mainly composed of cracks on the white line of the toe and side-wall which were often linked (Table 54). It was the greater proportion of these lesions in treatment D which resulted in the treatment differences. Side-wall and white line cracks were often hairline and minor. However, only two gilts did not have at least one claw with a crack which had developed sub-surface haemorrhaging and infection. In contrast, only four gilts displayed major erosion at the heel/toe junction on any claw.

Rate of Growth and Wear of the Hoof Horn

Some measurements were lost from both experiments due to the marks either growing out from, or being worn off the horn. Gilts from each treatment failed to contribute data to the final period (days 28 to 42) in particular, so that only a small sample was available.

Within treatments, the relationship between successive periods and the rate of growth was variable for both experiments (Tables 55 and 56). The first experiment (Table 55) showed an increase in the rate of growth between the first and second fourteen day periods, followed by a decrease in the third period, which was still at a higher rate than in period 1.

142

TABLE 49

Effect of treatment on percentage incidence of claws showing defects

| Treatment | Number of claws | | | | | | | | | Mean number of claws/gilt with defects |
	0	1	2	3	4	5	6	7	8	
A	12.5	25.0	25.0	37.5	0.0	0.0	0.0	0.0	0.0	1.9 ± 0.4
B	0.0	0.0	28.6	28.6	14.3	14.3	0.0	14.3	0.0	3.7 ± 0.7
D	0.0	25.0	25.0	0.0	25.0	12.5	12.5	0.0	0.0	3.1 ± 0.7
E	25.0	25.0	25.0	0.0	25.0	0.0	0.0	0.0	0.0	1.8 ± 0.6

143

TABLE 50

Effect of treatment on mean number of defects/claw

	Claw								
	Right Fore		Left Fore		Right Hind		Left Hind		
Treatment	Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner	Overall[1]
A	0.13	0.38	0.38	0.25	0.25	0.50	0.63	0.13	2.63
B	0.43	0.57	0.29	0.86	0.71	0.57	0.71	0.43	4.57
D	0.50	0.75	0.50	0.25	0.50	0.50	0.88	0.00	3.88
E	0.13	0.88	0.25	0.25	0.63	0.13	0.13	0.00	2.38

Note: All analysis by Chi-square.

[1] Treatments A, D and E were statistically significantly different from control Treatment B ($p < 0.001$). Treatments A and B, E and B were statistically significantly different for the mean number of defects/ sow ($p < 0.05$).

TABLE 51

Effect of treatment on the mean number of type of defect per region

| | Defect | | | | | |
| | Bruising and abrasion | | | | | Cut |
Treatment	Heel	Heel/toe	Toe	Side-wall	Combined regions	Heel
A	0.13	0.13	0.63	0.38	1.27	0.88
B	0.43	0.71	0.57	0.43	2.14	1.86
D	0.25	0.25	0.75	0.38	1.63	1.63
E	0.13	0.00	0.75	0.13	1.01	1.25

Note: 1) An insufficient number of observations were available for analysis by Chi-square for each region having bruising and abrasion. Therefore the results for the regions were combined to permit an analysis. An overall statistically significant difference was observed for treatments A, D and E compared to control treatment B ($p < 0.05$).

2) No statistically significant difference was analysed for the cuts on the heel.

TABLE 52

Effect of treatment on percentage incidence of claws showing lesions

Treatment	Number of claws									Mean number of claws/gilt with lesions[1]
	0	1	2	3	4	5	6	7	8	
A	0.0	0.0	0.0	12.5	75.0	0.0	12.5	0.0	0.0	4.1 ± 0.3
B	0.0	0.0	0.0	0.0	14.3	42.9	28.6	14.3	0.0	5.4 ± 0.4
D	0.0	0.0	0.0	0.0	25.0	0.0	50.0	25.0	0.0	5.8 ± 0.4
E	0.0	0.0	0.0	0.0	12.5	50.0	37.5	0.0	0.0	5.3 ± 0.3

[1]Treatment differences for mean number of claws/gilt with lesions statistically significant by one-way analysis of variance at $p < 0.05$. Treatment A is statistically significantly different from the other treatments $(p < 0.05)$.

TABLE 53

Effect of treatment on the mean number of lesions/claw

	Claw[2]								
	Right Fore		Left Fore		Right Hind		Left Hind		
Treatment	Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner	Overall[1]
A	4.00	1.13	3.00	0.00	3.50	0.38	4.50	0.25	16.75
B	3.29	0.71	2.43	2.00	2.86	1.57	3.57	0.86	17.29
D	4.25	0.38	3.50	1.50	3.38	1.38	4.75	1.38	20.50
E	3.00	0.38	3.25	0.13	3.38	1.00	3.50	1.25	15.88

Note: All analysis by Chi-square.

[1] No significant difference was observed for the mean number of lesions/sows between treatments A, D and E compared to control treatment B.

[2] Analysis was undertaken on combined inner and combined outer claws for fore and hind feet respectively:

i. The incidence of lesions was statistically significantly different between claws for each treatment ($p < 0.01$).

ii. Within treatments, the incidence of lesions was statistically significantly different on the fore outer compared to the fore inner claws (treatments A, D, E, $p < 0.001$; treatment B, $p < 0.01$) and on the hind outer compared to the hind inner (treatments A, B, D, E, $p < 0.001$).

TABLE 54

Effect of treatment on the mean number of type of lesion per region

| Treatment | Erosion and crack | | | Crack | | Overgrowth |
	Heel	Heel/toe	Toe (median)	Toe (white-line)	Side-wall	Heel
A	0.63	0.88	0.38	5.75	8.75	0.00
B	0.71	1.00	0.71	7.00	8.29	0.00
D	0.50	0.00	0.25	9.25	10.38	0.00
E	0.50	0.50	0.50	7.00	7.38	0.00

Note: 1) An insufficient number of observations were available for analysis by Chi-square for erosion and crack lesions in the heel, heel/toe and toe (median) regions. Consequently the heel and heel/toe regions were combined and the toe (median) region was combined with the toe (white-line) region for analysis. The results for Treatments A, D and E were compared to control Treatment B.

2) Overall, a statistically significant difference was observed between treatments for the combined heel and heel/toe regions ($p < 0.01$). Treatments D and B were also statistically significantly different for these combined regions ($p < 0.05$).

3) No statistically significant differences were analysed for the lesions on the side-wall and combined toe regions.

148

TABLE 55

Effect of treatment on the rate of change of growth and wear of hoof horn for the first experiment

		Growth										Wear									
		Fore				Hind				Overall		Fore				Hind				Overall	
		Inner		Outer		Inner		Outer				Inner		Outer		Inner		Outer			
Treatment	Period	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.
A	1	0.22	0.07	0.24	0.06	0.25	0.04	0.34	0.03	0.26	0.03	0.29	0.05	0.27	0.02[a]	0.25	0.00[b]	0.32	0.01	0.28	0.01
	2	0.38	0.06	0.45	0.08	0.34	0.04	0.47	0.03	0.41	0.03	0.18	0.04[c]	0.21	0.03	0.24	0.06	0.17	0.05[d]	0.20	0.02
	3	0.32	0.08	0.40	0.15	0.50	0.11	0.44	0.06	0.41	0.05	0.29	0.04	0.31	0.01[e]	0.27	0.06	0.46	0.10	0.33	0.03
B	1	0.30	0.03	0.21	0.09	0.24	0.03	0.31	0.04	0.26	0.03	0.20	0.01	0.19	0.02[a]	0.18	0.04[b]	0.20	0.05	0.19	0.02
	2	0.24	0.03	0.45	0.05	0.38	0.05	0.50	0.02	0.39	0.03	0.20	0.01[c]	0.27	0.04	0.20	0.02	0.44	0.07[d]	0.28	0.03
	3	0.49	0.05	0.23	0.02	0.35	0.03	0.36	0.02	0.37	0.03	0.22	0.01	0.25	0.00[e]	0.21	0.05	0.26	0.01	0.24	0.01
D	1	0.12	0.02	0.27	0.08	0.25	0.04	0.34	0.06	0.24	0.03	0.21	0.03	0.25	0.03[a]	0.24	0.02[b]	0.31	0.02	0.25	0.01
	2	0.51	0.06	0.53	0.05	0.32	0.05	0.48	0.03	0.46	0.03	0.30	0.03[c]	0.24	0.02	0.28	0.06	0.29	0.01[d]	0.28	0.02
	3	0.32	0.12	0.20	0.11	0.35	0.05	0.47	0.04	0.34	0.05	0.35	0.03	0.26	0.01[e]	0.17	0.07	0.42	0.05	0.30	0.03
E	1	0.18	0.04	0.26	0.06	0.37	0.06	0.36	0.05	0.29	0.03	0.22	0.04	0.30	0.03[a]	0.30	0.02[b]	0.28	0.03	0.27	0.02
	2	0.38	0.09	0.36	0.07	0.38	0.05	0.49	0.06	0.40	0.03	0.18	0.01[c]	0.13	0.03	0.20	0.01	0.22	0.02[d]	0.18	0.01
	3	0.34	0.11	0.32	0.11	0.36	0.08	0.28	0.06	0.32	0.04	0.31	0.02	0.40	0.04[e]	0.28	0.02	0.34	0.04	0.32	0.02

Note: 1) All units in mm/day.

2) Despite the fourteen day period between measurements, marks were lost, therefore sample size varied within and between treatments for each period and between each period.

3) One-way analysis of variance was undertaken between treatments on all data for each claw within each period. Means denoted by a superscript indicate a statistically significant difference between treatments:

$$a, b, c, d = p < 0.05$$
$$e = p < 0.01$$

TABLE 56

Effect of treatment on the rate of change of growth and wear
of hoof horn for the second experiment

Treatment	Period	Growth Fore mean	Growth Fore s.e.m.	Growth Hind mean	Growth Hind s.e.m.	Wear Fore mean	Wear Fore s.e.m.	Wear Hind mean	Wear Hind s.e.m.
A	1	0.39	0.03	0.39	0.07	0.31	0.04	0.27	0.10
	2	0.35	0.04	0.37	0.04	0.20	0.03	0.27	0.03
	3	0.18	0.04	0.23	0.02	0.32	0.05	0.25	0.03
B	1	0.35	0.07	0.35	0.06	0.26	0.03	0.30	0.02
	2	0.48	0.09	0.44	0.07	0.33	0.08	0.28	0.02
	3	0.22	0.04	0.28	0.04	0.37	0.03	0.30	0.04
D	1	0.36	0.05	0.32	0.05	0.30	0.03	0.29	0.01
	2	0.31	0.05	0.37	0.03	0.27	0.04	0.30	0.02
	3	0.42	0.08	0.37	0.05	0.28	0.01	0.26	0.04
E	1	0.33	0.06	0.35	0.02	0.28	0.03	0.28	0.03
	2	0.41	0.06	0.39	0.04	0.32	0.04	0.27	0.03
	3	0.27	0.06	0.30	0.04	0.24	0.03	0.26	0.02

Notes: 1) All units in mm/day.

2) Marks were lost from the hoof horn despite only fourteen days between measurements, therefore sample size varied for analysis within and between treatments for each period and between each period of growth and wear.

3) No statistically significant differences were observed by one-way analysis of variance: i) between treatments for each period; ii) for each period within a treatment; and between fore and hind feet.

The second experiment (Table 56) showed an overall decrease in the rate of growth by the final period except for treatment D in which there was a slight increase. The rate of wear showed no trend within treatments for each experiment.

The first experiment showed no treatment effects. No clear relationship developed in either experiment although there were statistically significant differences between treatments in the second. The fore outer and hind inner claws showed treatment B to be growing at the slowest rate in the first period (p <0.05). The fore inner claws showed treatment D to be growing at a faster rate than the other treatments during the second period (p <0.05) whereas the hind outer claw of treatment B was growing faster (p <0.01) for the same period. Faster growth was exhibited by the fore outer claw of treatment D during the third period (p <0.001).

The Durometer

Incomplete sets of data were taken from the toe region of some gilts as it proved to be difficult to use the instrument effectively on this small area. The side-wall region did not present such practical difficulties, but as the Durometer did not operate correctly over cracks, these readings were discarded.

Differences in measurements were found between the side-wall and toe regions and also the inner and outer claws. When treatments were combined, the side-wall of the inner claw showed significantly higher readings than the outer for both fore and hind claws (Table 57). Toe readings exhibited an opposite but non-significant trend. Higher readings were obtained from the side-wall than the toe regions. Generally, higher measurements were exhibited by treatment D and lower measurements by treatment A for the side-wall region (Table 58). Treatments B and E tended to be intermediate, which were highlighted by the fore and hind outer side-wall readings. Treatments B and D provided significantly higher measurements (p <0.05) than A for the side-wall of the outer fore claw (A = 70.5 vs B = 72.7, D = 73.7; s.e.d. = 0.9). Treatment D gave significantly higher measurements (p <0.05) than A and E for the side-wall of the outer hind claw (A = 71.4, E = 71.6 vs D = 73.3; s.e.d. = 0.8).

The toe regions had a more variable but similar trend. The outer regions

TABLE 57

Comparison of readings of toe and side-wall regions of claws using a Durometer

152

Claw	Region	Durometer Reading		s.e.d.	Level of significance
		Inner Claw	Outer Claw		
Fore	Side-wall	73.5	72.3	0.5	*
Fore	Toe	59.7	60.6	1.1	NS
Hind	Side-wall	73.9	72.3	0.4	***
Hind	Toe	57.4	59.4	1.2	NS

TABLE 58

Effect of treatment on Durometer readings of claws

Claw	Region	Treatment				s.e.d.	Level of significance of treatment effects	One-way analysis of variance (5% level)[1]
		A	B	D	E			
Fore	Side-wall	72.5	73.1	74.7	73.8	0.9	N.S.	
Inner	Toe	58.5	60.9	59.8	59.8	2.8	N.S.	
Fore	Side-wall	70.5	72.7	73.7	72.2	0.9	**	AEBD
Outer	Toe	58.6	62.0	62.4	59.4	2.7	N.S.	
Hind	Side-wall	73.8	74.4	74.1	73.6	0.9	N.S.	
Inner	Toe	51.7	56.3	61.2	66.6	1.4	***	ABDE
Hind	Side-wall	71.4	73.0	73.3	71.6	0.8	*	AEBD
Outer	Toe	61.6	58.8	61.8	55.4	1.7	**	ADBE

[1]Treatments underlined together are not statistically significantly different.

showed treatment A to have the lowest readings and treatment D the highest with B and E intermediate. Treatments B, D and E were significantly higher ($p < 0.05$) than A for the hind inner claw toe measurements (A = 51.7, B = 56.3, D = 61.2, E = 66.1, s.e.d. = 1.4). Treatments D and E also had significantly higher readings than treatment B ($p < 0.05$). However the hind outer toe exhibited a different pattern: treatments A and D were significantly higher ($p < 0.05$) than treatment E, with B intermediate.

Fatty Acid Studies

The composition of perinephric fatty acids was influenced by dietary biotin intake. There was a statistically significant increase in the proportion of saturated fatty acids (C16:0 + C18:0) to the monoene fatty acids (C16:1 + C18:1) with increased dietary biotin intake (Table 59). The individual fatty acids showed a similar statistically significant response in the neutral lipid fraction with treatment A being more unsaturated than the other treatments. The phospholipid fatty acids showed the same trend with only the C16:1 achieving statistical significance between treatments. A reduction of biotin intake during the growing period (treatment E) produced differential effects on the two lipid fractions. The monoene:saturated ratio showed that the composition of the netral lipid fraction was similar to that of pigs maintained on a high biotin intake throughout the trial (treatment D) whereas the phospholipid fraction had a fatty acid composition similar to that of pigs on a basal diet (treatment B; Table 62).

The phospholipid fraction was consistently more saturated than the neutral lipid fraction, in particular much high proportions of C18:0 and lower proportions of C18:1 were present for all treatments for both perinephric and hoof horn fat (Tables 59 and 60).

The fatty acid composition of the hoof horn fat was more unsaturated than the perinephric fat (Table 61). This resulted from a relative change in proportion of all the individual fatty acids except for C18:1 of the neutral lipid fraction. Notably, C16:1 was a significantly greater proportion of the total fatty acids present in both lipid fractions. Again, the phospholipid fraction of the hoof horn was more saturated than the neutral lipid fraction except for those pigs on the high biotin intake (treatment D) which displayed no difference between the lipid fractions. There were no statistically significant treatment

TABLE 59

The effect of dietary biotin intake on the percentage fatty acid composition of perinephric fat

Fatty acids		Treatments				s.e.d.	Level of significance of treatment effects	One-way analysis of variance (5% level)[1]
		A	B	D	E			
Perinephric fat; phospholipids								
C16:0		35.6	38.9	40.5	37.5	1.7	N.S.	
C16:1		6.9	3.8	2.1	3.4	0.9	***	ABDE
C18:0		21.4	24.6	27.7	26.8	2.8	N.S.	
C18:1		34.9	33.0	29.8	33.5	2.8	N.S.	
C16:0 + C18:0		58.2	63.5	68.1	63.1	3.2	*	DBEA
C16:1 + C18:1		41.8	36.7	31.8	37.0	3.2	*	DBEA
Perinephric fat; neutral lipids								
C16:0		28.8	33.0	33.6	33.6	1.2	***	ABDE
C16:1		9.6	4.0	5.4	4.4	0.8	***	ABDE
C18:0		7.1	10.9	12.1	12.1	1.4	**	ABDE
C18:1		54.7	52.1	49.1	50.1	2.1	*	ABDE
C16:0 + C18:0		35.8	44.0	45.7	45.6	2.0	***	ABDE
C16:1 + C18:1		64.2	56.1	54.4	54.4	2.0	***	ABDE
Level of	C16:0	**	**	***	**			
significance	C16:1	*	N.S.	**	N.S.			
between	C18:0	***	***	***	***			
phospholipids	C18:1	***	***	***	***			
and	C16:0 + C18:0	***	***	***	***			
neutral lipids	C16:1 + C18:1	***	***	***	***			

[1]Treatments underlined together are not statistically significantly different.

TABLE 60

The effect of dietary biotin intake on percentage fatty acid composition of hoof horn fat

Fatty acids		Treatment				s.e.d.	Level of significance of treatment effects
		A	B	D	E		
Hoof phospholipids							
C16:0		25.5	26.4	28.8	25.8	2.3	N.S.
C16:1		18.1	18.0	13.1	15.1	2.3	N.S.
C18:0		13.4	12.8	12.7	12.6	2.5	N.S.
C18:1		43.2	42.7	45.3	46.6	3.7	N.S.
C16:0 + C18:0		38.8	39.3	41.5	38.4	4.1	N.S.
C16:1 + C18:1		61.2	60.7	58.4	61.8	4.0	N.S.
Hoof neutral lipids							
C16:0		24.0	25.1	31.1	26.1	3.6	N.S.
C16:1		13.8	14.0	11.1	14.7	2.1	N.S.
C18:0		6.8	8.2	10.1	6.0	1.9	N.S.
C18:1		55.5	53.1	47.8	47.3	5.3	N.S.
C16:0 + C18:0		30.8	33.3	41.2	32.1	4.5	N.S.
C16:1 + C18:1		69.3	66.9	58.9	67.9	4.5	N.S.
Level of	C16:0	N.S.	N.S.	N.S.	N.S.		
significance	C16:1	N.S.	*	N.S.	N.S.		
between	C18:0	**	N.S.	N.S.	***		
phospholipids	C18:1	**	*	N.S.	N.S.		
and	C16:0 + C18:0	*	N.S.	N.S.	*		
neutral lipids	C16:1 + C18:1	*	N.S.	N.S.	*		

TABLE 61

Relationship between the perinephric and hoof fatty acids of the
phospholipid and neutral lipid fractions respectively for each treatment

Fatty acids	Perinephric fat Treatments				Hoof fat Treatments				Level of significance between fatty acids of perinephric and hoof fat			
	A	B	D	E	A	B	D	E	A	B	D	E
1) Phospholipids												
C16:0	35.6	38.9	40.5	37.5	25.5	26.4	28.8	25.8	***	***	***	***
C16:1	6.9	3.8	2.1	3.4	18.1	18.0	13.1	15.1	***	***	***	***
C18:0	21.4	24.6	27.7	26.8	13.4	12.8	12.7	12.6	**	***	***	**
C18:1	34.9	33.0	29.8	33.5	43.2	42.7	45.3	46.6	**	**	**	**
C16:0 + C18:0	58.2	63.5	68.1	63.1	38.8	39.3	41.5	38.4	***	***	***	***
C16:1 + C18:1	41.8	36.7	31.8	37.0	61.2	60.7	58.4	61.8	***	***	***	***
2) Neutral lipids												
C16:0	28.8	33.0	33.6	33.6	24.0	25.1	31.1	26.1	***	**	N.S.	***
C16:1	9.6	4.0	5.4	4.4	13.8	14.0	11.1	14.7	**	***	***	***
C18:0	7.1	10.9	12.1	12.1	6.8	8.2	10.1	6.0	N.S.	*	N.S.	***
C18:1	54.7	52.1	49.1	50.1	55.5	53.1	47.8	47.3	N.S.	N.S.	N.S.	N.S.
C16:0 + C18:0	35.8	44.0	45.7	45.6	30.8	33.3	41.2	32.1	**	**	N.S.	***
C16:1 + C18:1	64.2	56.1	54.4	54.4	69.3	66.9	58.9	67.9	**	***	N.S.	***

TABLE 62

Effect of treatment on the monoene:saturated ratio of fatty acids

	Treatment			
	A	B	D	E
Perinephric phospholipid fraction	0.72	0.58	0.47	0.59
Perinephric neutral lipid fraction	1.79	1.48	1.19	1.19
Hoof phospholipid fraction	1.58	1.54	1.41	1.61
Hoof neutral lipid fraction	2.25	2.01	1.43	2.11

effects although the same trend, as presented in the perinephric fats, existed in the hoof horn fat. The monoene:saturated ratio showed that a reduction of dietary biotin intake during the growing period (treatment E), resulted in a fatty acid composition similar to treatment A for the phospholipid fractions and intermediate to A and B for the neutral lipid fraction (Table 59).

DISCUSSION

At the first sampling, the gilts fed the diet containing egg-white (treatment A) had levels of plasma biotin which are considered to be indicative of biotin deficiency (Tagwerker, 1973), which agreed with the results of experiment 3. However, unlike experiment 3, samples from the older gilts showed an increased level of plasma biotin. Extra biotin may have been obtained by coprophagy as the pigs had access to their dung, however, coprophagy was not observed and it would be difficult to explain a difference between the two trials on this basis. Despite having higher plasma biotin values than pigs in experiment 3, the treatment A gilts did develop a rough hair coat visibly different from that of the gilts in the other treatment groups but these characteristic clinical symptoms of early biotin deficiency did not develop further. It has been previously reported (Cunha et al., 1968; Glättli et al., 1975) that other symptoms such as poor weight gain and reduced feed conversion efficiency usually occur before clinical symptoms are observed. However, the growth rate of the treatment A gilts was significantly greater than that of the gilts on the other dietary treatments which confirms the results obtained in experiment 3. This was explained by the extra energy and protein provided by the addition of 5% egg white to this diet.

It is also worth noting that the feed scales for experiments 3 and 5 were fed in order to minimise the variation of metabolic rate with body weight (W) by relating metabolic rate to W^b, the metabolic body size. The value of b was 0.75 which is commonly used for interspecific comparisons. More recently Brown and Mount (1982) have shown that for intraspecific use in the pig, a lower value of 0.6 is more appropriate. Hence an unexpected variation may have been introduced in these experiments and a higher level of biotin fed per kilogram metabolic body weight than originally intended.

159

The examination of the hooves prior to slaughter provided results which did not correspond with those described in experiment 1 or with the Durometer readings or fatty acid assessments obtained in this experiment. Surprisingly the level of defects and lesions was higher in these gilts than in those found at 170 days for gilts in experiment 1. Only in the number of claws with defects for treatments A and D could comparable results be obtained to the 170 day assessment in experiment 1. Otherwise more defects and lesions/claw and claws with lesions were recorded in the current trial. Generally more toe, side-wall, and heel out defects and less heel and heel/toe defects were recorded than at 170 days in experiment 1. The incidence of all categories of lesions recorded, in particular those of the toe white line and side-wall, were higher than found at 170 day assessment in experiment 1. In that experiment the main lesions were on the volar surface (heel and heel/toe junction). These lesions increased in experiment 1 in both treatments, but it was also the differences in rate of increase between treatments as the sow aged which was significant. Other workers have noted that a lessening in the severity of lesions associated with the heel and heel/toe occurred with biotin supplementation (Bujas et al., 1972, cited by Tagwerker, 1973; Brooks et al., 1977; Bryant et al., 1980; Penny et al., 1980). The observations of hoof horn damage in experiment 4 compared to experiment 1 provide further evidence that the relationship between dietary biotin intake, environment and hoof horn damage is complex and that hoof lesions alone may not give a complete explanation. The high number of side-wall lesions, including horizontal cracks, in all treatments in experiment 4 suggests that these may not be representative of biotin deficiency in the growing pig. In fact, despite the number of lesions noted, the hoof health of the pigs on all treatments was good with little apparent secondary infection and underrunning. These results highlight the need for a deeper understanding of the effect of dietary biotin intake on the physical parameters and biochemistry of the hoof.

The effect of treatment on growth and wear of the horn was variable. Unfortunately, even though the period between recordings was reduced to two weeks, hoof marks were still lost, particularly during the third two-week period. The rate of growth was generally comparable with the results of Geyer (1979) and that found in experiment 3. As expected in

a growing animal, the rate of wear was less than the rate of growth. The overall growth rate of treatment A was higher and the growth rate of the horn of this treatment may have been expected to be greater too. Difficulties arose in obtaining sufficiently accurate measurements to observe a treatment effect from a gilt's hoof which is a small structure at this age.

The treatment differences shown by the Durometer corresponded well with the plasma biotin results, which further suggests that structural changes were occurring even if growth rate were not affected. The lowest Durometer readings and therefore least hard hoof horn was displayed by the biotin-deficient treatment A. The hardest horn was shown by the treatment receiving the highest intake of biotin (treatment D); treatments B and E were intermediate. The measurements of the toe had more variation than the side-wall. It was difficult sometimes to rest the Durometer perpendicularly against the volar surface of the toe which introduced a degree of inaccuracy into the result. Such problems were not encountered with the side-wall. Although treatment A in experiment 3 also had the least firm horn, there were large differences between the absolute values for similar regions between experiments 3 and 4, with lower values for similar treatments in experiment 3. The relationship between DLWG and saturation of perinephric fat in experiments 3 and 4 may explain the different Durometer readings between those experiments. The main difference in method between treatments A, B and D in experiments 3 and 4 was the daily ration and not the diet.

It has been generally accepted that as the level of feed intake is increased, fat deposition increases and fatty acids become more saturated. Scott et al. (1981) found more saturated fatty acids from increased de novo fatty acid synthesis in the fat of obese swine. Conversely reduced feed levels have resulted in less de novo fatty acid synthesis and a preferential deposition of unsaturated dietary fatty acids (Callow, 1937) in which C18:1 often predominated (Hilditch et al., 1939; Dahl and Persson, 1965). Lea et al. (1970) also stated that faster growth tended to produce firmer adipose tissue but the animals in experiment 4, which showed a slower DLWG than those in experiment 3, had more saturated perinephric fat. The hoof fatty acids were not assessed in experiment 3 but may have been expected to show a similar

trend to the perinephric fat resulting in a more unsaturated fat. A higher level of unsaturation may be one of the factors which produces a softer hoof horn which could explain the lower Durometer readings in experiment 3. Certainly the Durometer can provide useful information on the firmness of hoof horn in a comparative study. However, before the instrument could be used in field assessments, more work is needed to achieve a full understanding of its relationship to hoof horn hardness.

Increasing saturation of depot fat has also been associated with increasing maturity (Sink et al., 1965), but this observation was made on pigs with a wider age range than in experiments 3 and 4. An increase in saturation that occurred after about 55 kg live-weight was attributed to a relative increase in C18:0 over C18:1 (Allen et al., 1967). Scott et al. (1981) also reported a tendency for increased saturated fatty acids in pigs from three to six months of age. Therefore the observed differences in fatty acid composition between treatments appeared to be unrelated to the growth performance of the pigs.

The effect of fat metabolism would appear to be associated with dietary biotin intake and occurred prior to the manifestation of clinical symptoms of biotin deficiency. It was highlighted by the change in monoene:saturated ratio for experiments 3 and 4 (Tables 46 and 62). This ratio is largely determined by fatty acid desaturase activity whereas the content of linoleic and higher polyenes is determined mainly by the intake of dietary fat (Lea et al., 1970). Pigs fed the biotin-deficient diet (treatment A) had the lowest proportions of saturated fatty acids in the perinephric sample and increasing dietary biotin intake resulted in an increasing proportion of saturated fatty acids. The monoene:saturate ratio of the hoof horn lipid showed a similar though non-significant treatment effect. These changes in the fatty acid profile of the hoof horn lipid may affect the permeability of the horn. This change may provide an explanation of field observations that the hoof horn of the biotin-deficient pig is more rubbery and prone to damage than the horn of animals on high biotin levels (Bühlmann, 1973; Comben, 1978).

The reduction in dietary biotin intake during the growing period

produced different responses in the neutral lipid and phospholipid fractions of the perinephric fat. These are two separate fatty acid pools. The phospholipid pool is associated predominantly with cell membranes and is less subject to change. Conversely, the neutral lipid pool is assumed to be in constant flux as it consists of unbound fatty acids. Therefore a greater response to dietary biotin might have been expected in the neutral lipid fraction rather than, as occurred, the phospholipid fraction. The response to a sudden reduction in dietary biotin intake by both fractions of horn lipid resulted in final levels of saturation closer to those of the biotin-deficient animals rather than those of the animals on the basal diet. These results parallel field observations in which biotin-deficient pigs recovered hoof health after being given very high levels of dietary biotin but when subsequently placed on a diet with adequate biotin, suffered a regression until they adjusted to the new dietary biotin regime.

The physical tests (compression yield strength reported in experiment 3 and the Durometer reported in experiment 4) suggest that some changes in hoof horn structure occur with level of dietary biotin intake. The results of experiments 3 and 4 also confirm that dietary biotin level affects the fatty acid composition of depot fat and at the same time produces changes in the composition of hoof fat. It still remains to be established whether these changes in hoof fat influence the physical characteristics of the hoof horn.

EXPERIMENT FIVE
THE EFFECT OF SUPPLEMENTING BREEDING SOW DIETS WITH BIOTIN ON THE FATTY ACID PROFILE OF PHOSPHOLIPIDS AND NEUTRAL LIPIDS OF DEPOT AND SOW MILK FAT IN EARLY AND LATE LACTATION

INTRODUCTION

Experiment 1 confirmed that the dietary intake of available biotin plays an important part in the reproductive performance of the sow. However, deciding whether the biotin status of a commercial animal is a constraint on its performance is still a problem as the techniques available for the analysis of the biotin status of the pig must be treated with caution. The most commonly-used measure is plasma biotin level, however this is only suitable as an indicator of the biotin status of groups of animals (Tagwerker, 1973). Plasma biotin determinations from suspectedly-deficient sows have indicated that biotin levels were higher in pregnant than in lactating sows, suggesting that the extent of deficiency may vary with reproductive status (P H Brooks, 1977, personal communication). Biotin demand is likely to be at its greatest during lactation for two reasons. Firstly, biotin is secreted in the milk and this in turn increases the dietary biotin requirement. Secondly, lactating sows usually catabolise fat. As there is a biotin requirement for fat catabolism, a marginal biotin supply may directly influence milk production. Milk fat composition reflects the changes in composition of plasma triglycerides (Witter and Rook, 1970) and so may respond rapidly to changes in the biotin status of the sow. Subcutaneous depot fat responds to long-term changes in dietary biotin and may not reflect transient changes in the biotin status of the animal.

The relationship between the long-term effect of the level of dietary

biotin intake and subcutaneous and milk fat could be investigated using the technique developed in experiments 3 and 4. These experiments demonstrated that a first stage fractionation of tissue lipids into phospholipids and neutral lipids produced results which showed a biochemical response to marginal differences in biotin supply. It was decided that information on the long-term effects of dietary biotin intake could be provided from sows in experiment 1 which had been on two different dietary biotin regimes for a minimum of eighteen months.

MATERIALS AND METHODS

Treatments

Two representative groups of nine and six sows from the control and supplemented treatments respectively of experiment 1 were sampled for milk and subcutaneous fat during their third or fourth lactations. The samples were taken within forty-eight hours and at thirty-four to thirty-six days following farrowing and the fatty acid composition of the phospholipids and neutral lipids present was determined. The dietary treatments and management procedure for these sows have been described in experiment 1.

Fat and Milk Samples

The fat and milk samples were taken within two days of parturition and at weaning.

Each sow was secured by a nose-tether. The udder was cleaned with warm water. Following the introduction of 10 I.U. of oxytocin into an ear vein, the milk was available immediately for collection and at least 100 ml of milk was collected by hand milking. The milk was sealed in jars and deep-frozen.

The shoulder region was then prepared for fat sampling. A local anaesthetic was applied to the region. Time was allowed for the anaesthetic to take effect, after which a 25 mm incision was made and a sample of subcutaneous fat of approximately one hundred cubic millimetres in dimension was excised. A single suture sealed the wound. At five weeks of lactation the procedure was repeated, a fat sample being taken from the opposite shoulder.

165

Biotin Assay of Milk Samples

At least 10 ml of each milk sample was set aside for biotin assay prior to deep-freezing. The assay technique was described by Frigg and Brubacher (1976).

Extraction of Fatty Acids from Neutral Lipids and Phospholipids of Subcutaneous Fat and Milk

1) Milk Samples

Following defrosting, extraction of fat from the milk was undertaken by the Rose-Gottlieb method as described below. Protein present within the sample was first dissolved in concentrated ammonia and the fat then extracted with ether and petroleum spirit.

About 10 g of milk was placed into a Rose-Gottlieb tube. 1 ml of 0.880 ammonia was added; the tube was stoppered and the contents mixed thoroughly. 10 ml of ethanol was introduced and again mixed well. 25 ml of washed ether was added and the whole shaken for 30 seconds. Finally, 25 ml of petroleum ether was added to the mixture and the tube was shaken for another 30 seconds. The vessel was left to stand for about twenty minutes by which time the upper layer had cleared; siphoning tubes were fitted and as much of the top fat solution as possible transferred to a clean flask.

The extraction was repeated twice more with 15 ml of each solvent each time. The lipid was recovered by removal of the bulk of the solvent by distillation; the solvent residue was evaporated under a jet of nitrogen.

The procedure at this point for the production of the methyl esters of fatty acids of phospholipids and neutral lipids was the same as that described for the hoof horn lipid in experiment three. The same four fatty acids were studied as described in experiments three and four.

2) Fat Samples

The procedures for fat extraction have been described previously (p 120).

166

Unfortunately fat and milk samples were not taken from all the designated sows at weaning as veterinary assistance was unavailable at all times. Other milk samples could not be obtained in sufficient quantities as some sows had developed mastitis. A failure in assay techniques also resulted in the loss of some of the data from shoulder fat for fatty acid analysis and milk for biotin content. As a result only fat samples of two sows from each treatment were fully analysed from the late lactation. These data were not statistically significant.

Only 3 and 5 control sows and 5 and 4 supplemented sows contributed data on milk biotin content pre-farrowing and at weaning respectively.

The biotin content of the milk of the supplemented sows was higher than the control sows (4269 $\pm$ 250 ng/100 ml vs 2693 $\pm$ 2274 ng/100 ml respectively) following farrowing (Table 63). The mean biotin content of the control sows at weaning was lower (957 $\pm$ 480 ng/100 ml). The supplemented sows showed an increase in milk biotin content (5111 $\pm$ 1624 ng/100 ml).

The neutral lipid fractions of the fatty acids from milk and shoulder fat were more unsaturated than their respective phospholipid fractions (Tables 64 to 67). This was shown in the milk samples by an increase in C18:1 and a proportional decrease of C16:0 for early and late lactation respectively. The shoulder fat samples showed a large increase in C18:1 as well, but a proportional decrease mainly of C18:0 for early and late lactation respectively (Tables 66 and 67). This was highlighted by the monoene:saturated ratio which also showed the shoulder fat to be more unsaturated than the milk fat. The major proportion of the fatty acids was provided by C16:0 from sow milk compared to the shoulder fat samples in which C18:1 was the major fatty acid. C18:0 contributed least to the fatty acids of the milk samples whereas C16:1 was only a minor proportion of the fatty acids from the shoulder fat samples.

The milk samples showed an increase in unsaturation in the phospholipid fraction for both treatments.

TABLE 63

Effect of treatment on biotin content of milk

| | Treatment | | | |
| | Control | | + Biotin | |
Period	mean	s.e.m.	mean	s.e.m.
Post-farrowing	2693	2274 (3)	4269	250 (5)
Weaning	957	480 (5)	5111	1624 (4)

Note: Figures in parentheses indicate sample size.

TABLE 64

Effect of treatment on the neutral lipid fraction of sow milk

Fatty acid	Early lactation			Late lactation		
	Control (9)	+ Biotin (6)	s.e.d.	Control (5)	+ Biotin (4)	s.e.d.
18:1	37.9	37.6	4.8	39.2	48.2	6.8
18:0	2.7	1.9	1.2	2.4	5.1	1.3
16:1	16.3	18.5	2.2	15.9	10.9	2.7
16:0	43.3	42.5[a]	3.5	42.1	35.9[a]	5.4
18:1 + 16:1	54.2	56.0	3.6	55.1	59.2	5.1
18:0 + 16:0	45.8	44.0	3.6	45.2	40.9	5.0
Monoene:saturated ratio	1.20	1.27		1.22	1.45	

Note: [a]Means with the same superscript differ at $p < 0.05$.

Figures in parentheses indicate sample size.

TABLE 65

Effect of treatment on the phospholipid fraction of sow milk

Fatty acid	Early lactation			Late lactation		
	Control (9)	+ Biotin (6)	s.e.d.	Control (5)	+ Biotin (4)	s.e.d.
18:1	30.4	30.8	5.1	34.3	35.9	6.4
18:0	5.5	4.4	1.3	4.3	3.4	1.5
16:1	13.4	14.1	1.8	14.1	12.7	3.2
16:0	50.8	50.7	4.7	47.2	45.7	6.3
18:1 + 16:1	43.8	44.8	5.1	48.5	51.0	5.3
18:0 + 16:0	56.2	55.2	5.3	51.5	49.0	5.3
Monoene:saturated ratio	0.78	0.81		0.94	1.04	

Note: Figures in parentheses indicate sample size.

TABLE 66

Effect of treatment on the neutral lipid fraction of the subcutaneous fat of the sow

Fatty acid	Early lactation			Late lactation		
	Control (9)	+ Biotin (5)	s.e.d.	Control (2)	+ Biotin (2)	s.e.d.
18:1	60.9	62.5	3.7	62.1	57.6	8.9
18:0	7.6	8.1	1.4	7.3	6.4	2.2
16:1	5.2	4.2	1.6	6.3	6.4	3.2
16:0	26.5	25.2	3.6	24.5	29.7	7.8
18:1 + 16:1	66.2	66.7	3.9	68.4	63.9	5.9
18:0 + 16:0	33.9	33.3	3.8	31.7	28.5	5.4
Monoene:saturated ratio	1.95	2.00		2.16	2.24	

Note: Figures in parentheses indicate sample size.

TABLE 67

Effect of treatment on the phospholipid fraction of the subcutaneous fat of the sow

Fatty acid	Early lactation			Late lactation		
	Control (9)	+ Biotin (5)	s.e.d.	Control (2)	+ Biotin (2)	s.e.d.
18:1	44.6	43.6	4.5	42.9	48.7	5.7
18:0	19.1	23.6	3.4	24.6	18.5	5.9
16:1	4.2	4.6	2.2	3.3	5.6	2.8
16:0	29.9	28.5	2.4	29.3	27.3	2.9
18:1 + 16:1	51.0	48.0	4.3	46.1	54.3	8.5
18:0 + 16:0	49.1	52.1	4.3	54.0	45.9	8.5
Monoene:saturated ratio	1.04	0.92		0.85	1.18	

Note: Figures in parentheses indicate sample size.

The control treatment showed no change in the level of unsaturation of the neutral lipid fraction from early to late lactation, whereas the supplemented treatment displayed an increase. This resulted from a large increase in C18:1 (48.2% vs 37.6% for early and late lactation respectively) and a large decrease ($p < 0.05$) in C16:0 (42.5% and 35.9% for early and late lactation respectively).

DISCUSSION

The reduction of the biotin content of the milk of the control sows is supported by data for the plasma biotin content of lactating sows in experiment 1, by P. H. Brooks (1977, personal communication) and Money and Laughton (1980). Biotin is being utilised during lactation at an increased level. Dietary biotin supplementation prevented the reduction occurring in milk as well as in the blood plasma (experiment 1).

Sow milk fat had a high proportion of C16:0 and C16:1 compared with subcutaneous shoulder fat and perinephric fat (experiments 3 and 4). The higher levels of C16:1 compared to sources of body fat was expected as a desaturase system converting C16:0 to C16:1 is present in the mammary gland (Witter and Rook, 1970). Unexpectedly, an increase in unsaturation was observed in both fatty acid fractions of the milk between the samples taken after parturition and before weaning. Previously an increase in saturation of the fatty acids during lactation has been described. The monoene:saturated ratio decreased between samples of colostrum and milk (deMan and Bowland, 1963) and between samples taken at seven and twenty-eight days following parturition (Bakke and Vold, 1975). Only a slight reduction in C18:1 was observed by Stahly et al., (1981) in samples taken at seven and twenty-one days following parturition. Colostrum resembled back fat more than did milk (deMan and Bowland, 1963). Tollerz and Lindberg (1965) also observed that the fat in the first few days seemed to resemble the depot fat more than did the later milk. They stated that most C16:0 and shorter acids were believed to be synthesised through a step-wise condensation of acetyl Co A units in the mammary gland. As this is biotin-mediated, should insufficient biotin be available, a decrease of C16:0 and C16:1 would be expected in later lactation. The relative proportion of C16:0 did decrease in both the phospholipid and

173

neutral lipid fraction, but in both treatments.

The C18:1 in milk fat arises from preformed C18:1 in the blood plasma which in turn comes from the digestive tract or from reserves in the organism (Tollerz and Lindberg, 1965). Therefore, although the level of C16:0 is usually considered constant (Flanzy et al., 1970), dietary fat will influence the composition of milk fat during lactation (deMan and Bowland, 1963; Rastogi, 1977; Stahly et al., 1981). This may explain the variation in proportions of fatty acids of unfractionated milk samples shown by several workers. The monoene:saturated ratio for milk fat can be calculated as 1.40, 1.32, 1.31, 1.22 and 1.07 from the data presented by Seerley et al. (1981), deMan and Bowland (1963), Melichar et al. (1973), Meyer et al. (1980) and Duncan and Garton (1966).

Clearly the proportions of fatty acids in milk are influenced by dietary factors other than biotin. The limited scale of the work makes it unwise to draw definite conclusions, but the treatment differences observed indicate the need for further more detailed studies on the relationship between dietary biotin intake and depot and milk fat composition.

174

CONCLUDING DISCUSSION

Brooks (1978) demonstrated that the increase in productive efficiency of the sow over the previous twenty years had resulted in less sow feed being consumed per weaner pig produced and a lower provision of biotin per kilogram produced. The source of dietary biotin may have been reduced further as a result of a change from maize to wheat and barley-based feeds which contained less available biotin (Comben, 1978). Some evidence of biotin-responsive conditions in sows on commercial pig units had been presented by Brooks et al. (1977) and Comben (1978). The work by Brooks et al. (1977) offered the first indication in a short-term controlled experiment that the provision of supplemental biotin in feed may reduce the level of damage to the hoof horn and improve the reproductive performance of sows suffering from a suspected deficiency of biotin prior to treatment. However, the origin of the biotin deficiency had not been ascertained. It was hypothesised that the amount of available biotin in the feed had not supported the optimum level of horn production or reproductive performance. To test this hypothesis further investigation was needed to assess the effects of low biotin intake on the long-term productivity of the sow.

The results reported in experiment 1 confirmed that sows fed diets with low levels of available biotin did not perform as well as those given supplementary dietary biotin. No other work published to date has investigated the long-term effect of low availability of dietary biotin on the hoof lesions of initially healthy stock from 25 kg liveweight. However in other experiments which commenced with initially healthy stock, biotin supplementation has also been shown to improve the hoof integrity of young sows (Grandhi and Strain, 1980; Bryant et al., 1982). The amount of hoof damage in stock having an initial high incidence of hoof lesions was reduced by dietary biotin supplementation following four months on treatment (Triebel and Lobsiger, 1979; Money and Laughton, 1980; De Jong and Sytsema, 1983) and in only four weeks when biotin was

175

provided by intramuscular injection as well (Bujas et al., 1972, cited by Tagwerker, 1974). Although the damage to the hoof horn was not reduced in the herd investigated by Penny et al. (1980) following biotin supplementation, the authors did find that supplementary biotin afforded initially healthy replacement gilts a degree of protection from hoof horn damage. In experiment 1 the protective function of early biotin supplementation on hoof integrity was retained as the sow aged, although the differences between the supplemented and unsupplemented treatments were least following the fourth weaning when compared with assessments made following earlier weanings. The work of Michel and Mastachi (1981) showed no response to dietary biotin supplementation in older sows which were considered to be initially healthy. It can therefore be concluded that provision of a diet containing adequate available biotin from an early age affords the sow some long-term protection against hoof damage.

The mechanism by which biotin provides protection is still unresolved, although some effects of the level of dietary biotin intake on certain physical and biochemical parameters of hoof horn have been exposed in experiments 3 and 4 reported here. Physical tests using the Instron Materials Testing Instrument gave highly variable results, partly because it was difficult to produce samples of hoof horn of sufficiently large size to undertake the compression yield test. More notable results were obtained from the analysis of the fatty acid profile and from the measurement of hoof horn hardness using the Durometer. Higher levels of unsaturation were measured from perinephric and hoof horn fat of gilts given low available biotin diets. A more unsaturated fat may produce a softer hoof horn, certainly the physical test using the Durometer gave lower values for pigs with more unsaturated fat samples. As the Durometer measurement has not been related to an absolute value for the "strength" of the hoof horn, caution must be exercised in drawing conclusions from the comparisons of the results from the Durometer and the fatty acid analyses. A better understanding of this relationship might be obtained if investigations of fatty acid profiles and Durometer measurements were undertaken on the larger hoof horn of the sow from which sufficient samples could be taken for the compression yield test to give a measure of strength. If these values correlated with Durometer readings, the Durometer could provide a practical and non-destructive

technique for assessing the condition of the hoof horn of a live pig and, possibly, relating it to its biotin status.

The effect of the level of dietary biotin intake on hoof lesions in experiment 1 was unrelated to the effect on reproductive performance. Although biotin supplementation reduced the number of lesions suffered by the sows compared with the control group, there was no greater incidence of culling for foot damage nor were any general signs of undue suffering exhibited by the control group. This is in contrast to experiments in which lameness was a severe problem prior to treatment. In such instances, the effect on reproductive performance of feeding biotin-supplemented and unsupplemented diets may still have been related to hoof condition as lameness or tenderness of the hoof could have affected mating behaviour (Brooks et al., 1977; Penny et al., 1981; Pedersen and Udesen, 1980). Therefore the experiments of Brooks et al. (1977) and Penny et al. (1981), in which statistically significant improvements in litter size and post-weaning performance were obtained as a result of dietary biotin supplementation, must be treated with caution. However it is also noteworthy that these experiments provided levels of 74 µg available biotin/kg or less in the unsupplemented feeds. In other studies on litter size a statistically significant improvement was achieved by the biotin supplementation of a basal diet of 74 µg available biotin/kg (Michel and Mastachi, 1981) and of under 110 µg available biotin/kg (Tribble, 1983). The work reported by Pedersen and Udesen (1980) and in experiment 1 also had a trend towards higher litter numbers with supplementation of a basal diet providing less than 75 µg available biotin/kg. Other workers (Easter et al., 1979; Robres Serrano and Garcia de la Calera, 1981; Bryant et al., 1982), starting with healthy stock, have also obtained a similar improvement in litter size following dietary biotin supplementation. These authors provided basal diets with higher levels of available biotin than in the previously described trials. Only Grandhi and Strain (1980) reported no differences in litter size or post-weaning performance. It was considered that the duration of this trial might have been too short for treatment differences in reproductive parameters to occur.

A statistically significant reduction in the weaning to conception interval was obtained as a result of dietary biotin supplementation of

177

initially healthy stock when the basal diet provided a low level of available dietary biotin (experiment 1). The weaning to remating interval was also significantly reduced by biotin supplementation in work reported by Bryant et al. (1982) who fed two basal diets containing a low level of biotin. A similar but non-significant trend was observed by Halama (1979), Robres Serrano and Garcia de la Calera (1981), Hamilton et al. (1983) and Tribble (1983).

These results suggest a complex relationship between herd health, available biotin content of basal diets and reproductive performance. There is some indication that in a herd which has been suffering from lameness which is responsive to dietary biotin supplementation, a significant improvement in reproductive performance is likely. It also appears that responses to reproductive parameters are more likely to occur when the basal diet provides a low level of available dietary biotin even when clinical symptoms of biotin deficiency are not present. The effect on reproductive performance of using feedstuffs with low biotin availability has been demonstrated by Bryant et al. (1981) who showed a small improvement in days to oestrus and conception rate when diets were formulated with maize and soya compared to wheat, which had a lower level of available biotin (an estimated 99 and 45 µg available biotin/kg respectively).

Most trials that have been reported, have shown an improvement in reproductive performance with supplemental dietary biotin but statistical significance was difficult to achieve. Clearly, any effects on reproductive performance may be modified by other factors, such as environment and stockmanship. Consequently, either a very large number of animals or very careful control of other variables is necessary in order to achieve statistically significant differences. More conclusive evidence that biotin status influences reproduction may be obtained by inducing biotin deficiency in sows. Work in progress applying this approach, appears to be confirming that biotin deficiency reduces litter size and increases the period from weaning to remating and conception (Misir and Blair, 1983). Because of the difficulty of statistically proving the effect of biotin supplementation on individual parameters of reproductive performance, an assessment of overall productivity would be more useful. For instance, the output of a sow over her lifetime in terms

of total weight of weaner produced in unit time, would result from a com-
bination of all reproductive parameters. This approach was used to
analyse data in experiment 1 and showed the advantage of biotin supp-
lementation to the sow (Table 23). It also enables the potential cost
benefit of providing biotin in a diet to be assessed.

It is not possible to provide an explanation for the effect of biotin
on the reproductive metabolism of a sow. Biotin is involved directly
and indirectly in energy, fatty acid and protein metabolism and
inadequate levels of biotin may produce an energy-sparing effect but
this has not been confirmed in any investigation. It is also possible
that the role of biotin in litter performance may be mediated through
fatty acid metabolism. A major fatty acid component of the sow's
uterus is arachidonic acid (Friend and Elliot, 1978) which is a known
precursor for prostaglandins. Biotin is necessary for the synthesis
of malonyl Co A which controls the transformation of arachidonic acid.
If this reaction were responsive to dietary biotin levels, it could
have an important effect on the reproductive physiology of the sow.
Little is known about prostaglandin effect on reproduction, so caution
must be exercised in hypothesising a mode of action. Nevertheless it
is noteworthy that one effect of inadequate dietary biotin may be to
limit the growth of the uterus, which in turn could reduce the number of
piglets the sow is capable of maintaining to term (experiment 2).
Furthermore the significant effects on reproductive performance have
not occurred until after the first litter has been weaned (Penny et al.,
1981; Tribble, 1983; experiment 1). Other evidence has suggested that a
greater demand for biotin has occurred during lactation; there was some
indication that the biotin levels in blood plasma (experiment 1) and
milk plasma (experiment 5) declined during lactation in the control
group but not in the biotin-supplemented group. If biotin demand is
increased as a result of lactation, an effect on uterine physiology
and growth may ensue following weaning. A consequent reduction in
uterine growth may have an effect on second litter size. However it
must be borne in mind that biotin is likely to have a more complex role
in the physiology of reproduction, of which effects on the uterus may be
only one factor.

Not only an improvement in piglet weight (Michel and Mastachi, 1980;

Tribble, 1983) but also a reduction in preweaning piglet mortality has been recorded as a result of biotin supplementation (Easter et al., 1979; Pedersen and Udesen, 1980). The amount of biotin supplied in the milk may affect these factors. Little is known about the importance of providing cofactors in milk for suckling pig growth, but as milk is the only source of nutrient for the piglet until creep feeding commences, an inadequate dietary supply of any nutrient would have a significant effect on the piglet. Another way in which the level of dietary biotin fed to the sow may influence the suckling piglet, may be through the composition of milk fat. Although the significance of changes in sow's depot fat composition (experiment 5) has not been elucidated, these changes may influence the mobilisation and availability of energy in terms of milk fat quality and/or quantity. Clearly these factors are important in piglet survival and growth and require further investigation.

That the level of dietary biotin intake influences the fatty acid profile of depot fat was confirmed in experiments (3 and 4) on the growing pig. The effect of biotin supply was also shown by the low plasma biotin levels of pigs on treatment A (egg-white supplemented) even though DLWG and FCR were not reduced. Although usual early symptoms of biotin deficiency were not generally apparent, some indications of a rough hair coat were observed in the treatment A gilts of experiment 4, but but not of experiment 3. However following fractionation of depot fat into neutral lipids and phospholipids, a greater proportion of unsaturated fatty acids was observed as available dietary biotin was decreased in both experiments. Bühlmann (1973) had previously demonstrated that an increase in unsaturation of fatty acids occurred in the analyses of unfractionated fat but these effects were associated with symptoms of extreme experimentally-induced biotin deficiency. There was no suggestion that changes in the degree of saturation of the fatty acids of adipose tissue occurred prior to other symptoms of biotin deficiency. Hence, analysis of unfractionated fat may be a less sensitive technique in assessing the changes in fatty acid proportions of depot fat compared to the fractionation into its neutral lipid and phospholipid components. The results in experiment 3, from both methods of analysis, provide evidence in support of this claim in which a response from the unfractionated fat analysis was observed only in treatment A, by an increase in the

proportions of unsaturated fatty acids.

Experiments 3 and 4 demonstrated that the level of dietary biotin intake had an effect on the proportions of all the fatty acids examined, whereas Flanzy et al. (1970) and Rerat (1972) stated that the level of palmitic acid in fat deposits was almost constant and was not influenced by feed. The concentrations of stearic and oleic acids were influenced by diet in depot fat (Rerat, 1972) and in milk fat (Tollerz and Lindberg, 1965). However the level of palmitic acid was affected by dietary fat intake in milk fat (Tollerz and Lindberg, 1965). These authors observed that an increase of C18 acids in milk as a result of the supply from the feed, was counterbalanced by a decrease of C14 and C16 acids - but these were relative changes in proportions. The question remains whether the influence of biotin on palmitic acid of depot fat altered its relative or absolute value. If the absolute value was changed, this technique may be diagnostic of the biotin status of a pig. The increase in proportion of palmitoleic acid when insufficient dietary biotin was provided was also an interesting phenomenon and merits further investigation. However the response of the fatty acids to dietary biotin intake probably occurred as fat was formed by de novo synthesis. It might be anticipated that biotin supplementation of present commercial feeds which have a high content of unsaturated oils, would show less response as most of the depot fat would be derived from dietary fat rather than as a result of de novo synthesis. Depot fat can be influenced by other factors, many of which have been investigated by other workers. Temperature (Dean and Hilditch, 1933), level of energy intake (Callow, 1933) and composition of dietary fat (Ellis et al., 1974) can all change the relative proportions of the constituent fatty acids. The differences in the proportion of saturation of the fatty acids between experiments 3 and 4 was explained by the difference in daily biotin intake in otherwise similar treatments. The breed of the pig can also influence the degree of saturation; for instance, Pietrain pigs have higher concentrations of unsaturated fatty acids compared to Large Whites (Wood, 1973). Between sexes within breeds, further variation in the composition of fat has been observed; boars have a significantly higher proportion of total unsaturated fatty acids compared to castrates, gilts being intermediate to both groups (Smithard et al., 1980).

Further clarification is required, therefore, of the relationship between the effects of biotin on the fatty acid profile of the pig and these other factors.

The use of the fatty acid assay of an initial fractionation of adipose fat as a diagnostic tool for assessing the biotin status of the pig, possibly through the level of palmitic acid measured, would be of great advantage. Certainly the accuracy of the microbiological biotin assay technique and the biological variability of animals calls into question the validity of the result it provides for feedstuffs and blood plasma. In fact, Tagwerker (1973) warned that the technique was only of use in assessing the biotin status of groups of pigs. The fatty acid assay has also shown itself to have a particularly sensitive response to dietary biotin intake, with changes in fatty acid profile occurring prior to other physical symptoms of biotin deficiency. The technique has permitted further confirmation of Comben's (1978) assertions that the availability of dietary biotin to the pig may have been reduced as a consequence of a change from maize to cereal-based diets. Work undertaken by Nishimuta et al. (1980) has shown that provision of a high level of triticale as a replacement for maize in diets for pigs, can result in suspected biotin deficiency from examination of the fatty acid profiles of the back fat. Five diets, none with biotin supplementation, were fed, one without triticale and four with different levels of replacement of triticale for maize. The results show that as more triticale replaced maize in the diets, the proportion of unsaturated fats increased, which was clearly displayed by the monoene:saturated ratio (Table 68). A comparison with the whole fat analysis in experiment 3 (Table 45) reveals that the diets with the higher levels of triticale provided a degree of unsaturation of fats equivalent to the egg white treatment which was considered to be biotin deficient. Nishimuta et al. (1980) did not consider the effect of the level of dietary biotin intake on their results and did not measure the biotin levels of the feed or stock. However, they did observe no significant effects in terms of performance or meat quality in feeding high levels of triticale to growing pigs, but such an inadequate level of dietary biotin could certainly have influenced the long-term productivity of a sow.

TABLE 68

Fatty acid profile of backfat (% of total) for diets containing maize and triticale[†]

Fatty Acid	Per cent maize replaced by triticale				
	0	20	40	60	80
C16:0	29.0	24.6	23.1	24.3	22.6
C16:1	3.3	4.4	5.0	5.0	6.1
C18:0	17.0	14.2	13.3	13.0	11.5
C18:1	50.7	56.8	58.3	57.7	59.9
C16:0 + C18:0	46.0	38.8	36.4	37.3	34.1
C16:1 + C18:1	54.0	61.5	63.3	62.7	66.0
Monoene:saturated ratio	1.2	1.6	1.7	1.7	1.9

[†]Nishimuta et al. (1980)

Note: The percentage data has been recalculated against 100% as other fatty acids have been removed from the table. The relative proportions between the fatty acids are the same and so permit comparisons with the data presented in experiment 3.

183

The design of experiment 1 did not permit any conclusions on the biotin requirement of the sow although biologically and economically significant improvements in sow productivity were achieved with biotin supplementation. Brooks (1978) extrapolated from work on poultry and suggested that the dietary requirement of the sow was probably within the range of 140 to 540 µg biologically available biotin/kg of feed. Without supplementation the majority of European diets would provide less biotin than the lower of these figures. The experimental evidence suggests that the lower the level of available biotin provided in a diet, the greater the effect on reproductive performance. The experimental evidence to date suggests that some improvement in reproductive performance could be obtained by supplementation of cereal-based diets with biotin. Notable improvements of reproductive performance could also occur in a herd which has been previously suffering from a problem of lameness which is responsive to dietary biotin supplementation. There is sufficient evidence to suggest that 140 µg available biotin/kg is inadequate to support maximum reproductive performance. Robres Serrano and Garcia de la Calera (1981) obtained a 4.3% increase in litter size when basal diets containing 103 to 155 µg available biotin/kg were supplemented with 200 µg biotin/kg. However this improvement was not statistically significant. Michel and Mastachi (1981) added either 100 or 200 µg biotin/kg to basal diets containing approximately 74 µg available biotin/kg and found significantly more piglets born to the gilts fed the higher level of supplementation. These results suggest that the requirement level probably exceeds 175 µg available biotin/kg. Finally it should be noted that in the trial of Penny et al. (1981) supplementation of a basal diet, calculated to provide 56 µg available biotin/kg, produced significant improvements in litter productivity. The daily supplementation allowances in that trial were 1160 µg biotin/sow/day in pregnancy and 2320 µg biotin/sow/day in lactation; respectively 64% and 18% higher than in experiment 1.

Experiments 2 and 3 showed that the pig is sensitive to and responds to levels of dietary biotin intake which have been previously considered to be sufficient. Biochemical responses were observed to occur prior to the appearance of traditional symptoms of biotin deficiency in the growing pig. In addition, the results of Penny et al. (1980), De Jong

and Sytsema (1982) and experiment 1 suggest that biotin supplementation of the growing pig will have a preventative effect on the degree of hoof horn damage. However reproductive effects tend to appear following the first lactation (Penny et al., 1981; Tribble, 1983; experiment 1) which suggests that although a requirement in excess of 175 µg available biotin/kg may be necessary for most of a sow's lifetime, higher levels may be indicated particularly during lactation and postweaning.

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The effect of different dietary protein levels following weaning on the subsequent reproductive performance of sows

INTRODUCTION

The nutrient requirements of the female pig during pregnancy and lactation have been investigated in numerous studies (ARC, 1983). Weaning to remating interval and litter size appear to be little affected by nutritional variation during these stages of the reproductive cycle (Elsley, 1972; Brooks and Cole, 1971). However, the level of nutrient intake between weaning and remating can affect subsequent reproductive performance. Gilts weaned from their first litter showed a progressive reduction in mean weaning to remating interval, range of interval and of animals exhibiting oestrus as post-weaning feed intake was increased. More pigs were also produced as the plane of nutrition increased (Brooks and Cole, 1972). However, no response was shown to post-weaning feeding levels in older sows (Brooks et al., 1974). Only feed levels were studied in these trials. The contribution of the protein fraction was not investigated. Low protein levels have prolonged the weaning to oestrus period (Svajgr et al., 1972). Brooks and Smith (1980) found no difference in weaning to remating interval between sows fed 184, 253 or 270 g protein/day post-weaning but there was a slight improvement in ovulation rate with increasing protein level.

Elsley and MacPherson (1972) reported that the nutritional requirements of the sows could be most efficiently met by the provision of two diets of differing protein concentration, one of which was fed in pregnancy and the other in lactation. However, it was not clear whether the change from a high protein lactation diet to a lower protein gestation diet would have an effect on future reproductive performance if made immediately on weaning or delayed until after conception. The experiment reported here investigated the effect of feeding a high protein lactation diet and a lower protein gestation diet during the weaning to remating interval on subsequent reproductive performance. This experiment was undertaken prior to the main experimental programme.

MATERIALS AND METHODS

Twenty-three pairs of sows of the same parity for each pair and weaned at the same time, were randomly allocated to either:

Diet A) 12.38% CP diet fed at 3.66 kg/sow/day; or
Diet B) 14.38% CP diet fed at 3.50 kg/sow/day.

Diet A contained cereals and soya and provided 453 g CP/day. Diet B also included fish meal and provided 503 g CP/day.

The feeding levels ensured that both diets provided the same daily intake of energy (46 MJ DE/day). The composition and calculated analyses of the diets are given in table 69.

The feed levels, management and housing of the sows were as described in experiment 1.

RESULTS

Fifteen sows were removed from the trial for management and health reasons. Three sows were anoestrus and culled. Other sows were culled through lameness, injury and failure to remain in-pig. Veterinary advice suggested that culling was not related to treatment but, as a result, the numbers remaining in each treatment by parturition were small.

The distribution of oestrus attainment was not significantly different for treatments A and B (8.45 $\pm$ 1.81 and 11.29 $\pm$ 2.82 days respectively, Table 70). No statistically significant difference was shown between treatments for number and weight of piglets born.

CONCLUSIONS

The feed levels of the treatments in this experiment were comparable with the high levels fed in previous trials. Reproductive performance of gilts but not sows was demonstrated to respond to the high feeding levels following weaning (Brooks and Cole, 1972; Brooks et al., 1975). The level of protein fed in both treatments was higher than that which elicited a response in previous work (Svajgr et al., 1972; Brooks and Smith, 1980). The results of this experiment show that even though

TABLE 69

Composition and calculated analysis of diets

	Diet A (%)	Diet B (%)
Barley	90.00	42.85
Wheat	-	45.23
Soyabean	7.5	4.76
Fishmeal	-	4.78
Betamix	2.5	2.38

In addition 25 g/tonne Rovimix H was added to each diet. Rovimix H contains 1% biotin.

Calculated analysis

Oil	1.88	2.00
Protein	12.38	14.38
Fibre	4.91	3.76
Lysine	0.55	0.68
Threonine	0.42	0.47
Methionine & Cystine	0.43	0.51
Calcium	0.11	0.37
Salt	0.81	0.21
Phosphorus	0.41	0.50
DE	12.54 MJ DE/kg	13.22 MJ DE/kg

Mineral/vitamin supplement provided per kg diet was as described in experiment 1 (Table 11).

3

TABLE 70

Effect of level of dietary protein fed during weaning to oestrus period on sow reproductive performance

Parameter	Diet A	Diet B
Number of sows	22	19
Days to oestrus	8.5 ± 1.8	11.3 ± 2.9
Number of sows	14	12
Number of pigs born	10.7 ± 0.4	10.8 ± 0.7
Number of pigs born live	10.6 ± 0.4	10.3 ± 0.7

Treatment A provided a lower level of crude protein and essential amino-acids, they were probably in excess of the requirements for reproduction. Hence, the feeding of either a higher protein lactation diet or a lower protein gestation diet during the weaning to remating period does not seem to be significant, providing a high level of feed is given.

FOOT STUDIES

PIG NO. _____ DATE _____ TRIAL NO _____

L   M M   L

LEFT
FRONT

RIGHT
FRONT

L   M M   L

LEFT
HIND

RIGHT
HIND
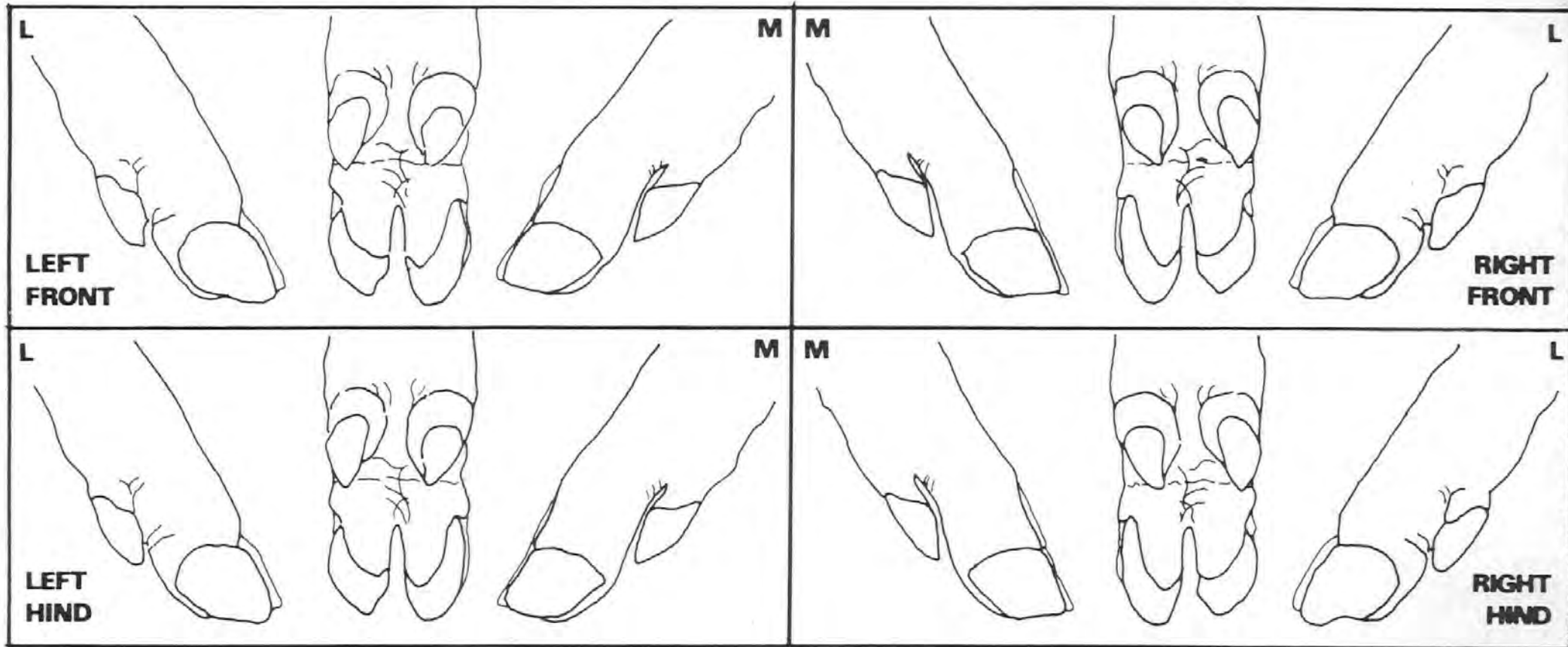
REMARKS. _____

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APPENDIX 3

Effect of dietary biotin supplementation and age of sow on the percentage

incidence of claws showing defects per sow

Period of examination	Treatment	Number of claws									Mean number of claws / sow with defects
		0	1	2	3	4	5	6	7	8	
170 days	Control (38)	23.7	26.3	28.9	5.3	7.9	0.0	5.3	2.6	0.0	1.8 ± 0.3
	+ Biotin (32)	25.6	33.3	25.6	7.7	2.6	2.6	2.6	0.0	0.0	1.5 ± 0.2
First weaning	Control (36)	13.9	19.4	13.9	22.2	13.9	13.9	2.8	0.0	0.0	2.6 ± 0.3
	+ Biotin (32)	6.3	25.0	31.3	6.3	18.8	3.1	3.1	3.1	3.1	2.6 ± 0.3
Second weaning	Control (31)	12.9	16.1	25.8	25.8	6.5	9.7	0.0	3.2	0.0	2.4 ± 0.3
	+ Biotin (29)	17.2	20.7	13.8	10.3	13.8	24.1	0.0	0.0	0.0	2.6 ± 0.3
Third weaning	Control (27)	11.1	18.5	37.0	18.5	14.8	0.0	0.0	0.0	0.0	2.1 ± 0.2
	+ Biotin (22)	18.2	22.7	31.8	18.2	9.1	0.0	0.0	0.0	0.0	1.8 ± 0.3
Fourth weaning	Control (24)	16.7	29.2	20.8	25.0	8.3	0.0	0.0	0.0	0.0	1.8 ± 0.3
	+ Biotin (21)	28.6	4.8	38.1	19.1	4.8	4.8	0.0	0.0	0.0	1.8 ± 0.3

Note: Figures in parentheses indicate sample size.

APPENDIX 4

Effect of dietary biotin intake and age of sow on the percentage
incidence of sows having defects for each claw

		Left Fore		Right Fore		Left Hind		Right Hind		
Period of examination	Treatment	Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner	Overall
170 days	Control (38)	34.2	23.7	28.9	26.3	23.7	18.4	15.8	13.2	23.0
	+ Biotin (39)	10.3	12.8	20.6	20.6	25.6	17.9	17.9	20.6	18.3
First weaning	Control (36)	41.7	36.1	25.0	47.2	33.3	22.2	41.7	16.7	33.0
	+ Biotin (32)	37.5	18.8	50.0	21.9	37.5	12.5	59.4	18.8	32.0
Second weaning	Control (31)	35.5	25.8	29.0	29.0	51.6	16.1	35.5	19.4	30.2
	+ Biotin (29)	41.4	27.6	37.9	27.6	41.4	37.9	37.9	24.1	34.5
Third weaning	Control (27)	25.9	40.7	33.3	22.2	25.9	18.5	25.9	18.5	26.4
	+ Biotin (22)	18.2	27.3	18.2	27.3	22.7	18.2	27.3	13.6	21.6
Fourth weaning	Control (24)	19.1	19.1	42.9	19.1	23.8	23.8	33.3	33.3	23.4
	+ Biotin (21)	14.3	19.0	23.8	28.6	28.6	9.5	12.5	19.0	22.6

Note: Figures in parentheses indicate sample size.

APPENDIX 5

Effect of dietary biotin supplementation and age of sow on mean number
of defects/claw

Period of examination	Treatment	Right Fore		Left Fore		Right Hind		Left Hind		Overall
		Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner	
170 days	Control (38)	0.42	0.26	0.29	0.32	0.26	0.21	0.16	0.16	1.87
	+ Biotin (39)	0.10	0.13	0.28	0.23	0.28	0.18	0.23	0.21	1.64
First weaning	Control (36)	0.50	0.39	0.28	0.50	0.39	0.25	0.47	0.19	2.97
	+ Biotin (32)	0.59	0.22	0.69	0.22	0.41	0.16	0.88	0.22	3.38
Second weaning	Control (31)	0.35	0.32	0.42	0.29	0.58	0.19	0.42	0.23	2.80
	+ Biotin (29)	0.62	0.34	0.38	0.28	0.45	0.03	0.52	0.34	2.96
Third weaning	Control (27)	0.30	0.48	0.41	0.22	0.26	0.19	0.30	0.19	2.35
	+ Biotin (22)	0.18	0.32	0.18	0.32	0.23	0.18	0.32	0.14	1.87
Fourth weaning	Control (24)	0.19	0.19	0.62	0.19	0.29	0.24	0.33	0.33	2.38
	+ Biotin (21)	0.14	0.24	0.29	0.29	0.29	0.10	0.43	0.19	1.97

Note: Figures in parentheses indicate sample size.

Effect of dietary biotin supplementation and age of sow on the percentage
incidence of claws showing lesions per sow

Period of examination	Treatment	Number of claws									Mean number of claws / sow with lesions
		0	1	2	3	4	5	6	7	8	
170 days	Control (38)	13.2	15.8	26.3	10.5	13.1	2.6	10.5	7.9	0.0	2.8 ± 0.3
	+ Biotin (39)	15.4	12.8	23.1	10.3	15.4	12.8	10.3	0.0	0.0	2.8 ± 0.3
First weaning	Control (36)	2.8	0.0	2.8	5.6	19.4	19.4	19.4	19.4	11.1	5.4 ± 0.3
	+ Biotin (32)	0.0	6.3	3.1	21.9	21.9	15.6	15.6	12.5	3.1	4.5 ± 0.3
Second weaning	Control (31)	0.0	0.0	3.2	3.2	16.1	22.6	12.9	25.8	16.1	5.8 ± 0.3
	+ Biotin (29)	0.0	6.9	3.5	10.4	10.4	20.7	10.4	13.8	24.1	5.4 ± 0.4
Third weaning	Control (27)	0.0	0.0	0.0	0.0	14.8	7.4	26.0	22.2	29.6	6.4 ± 0.3
	+ Biotin (22)	0.0	4.6	4.6	0.0	9.1	22.7	22.7	18.2	18.2	5.7 ± 0.4
Fourth weaning	Control (24)	0.0	0.0	0.0	4.2	16.7	8.3	25.0	16.7	29.2	6.2 ± 0.3
	+ Biotin (21)	0.0	0.0	0.0	4.8	14.3	28.6	19.1	9.5	23.8	5.9 ± 0.3

Note: Figures in parentheses indicate sample size.

Effect of dietary biotin supplementation and age of sow on the percentage
incidence of sows having lesions for each claw

Period of examination	Treatment	Claw								Overall
		Left Fore		Right Fore		Left Hind		Right Hind		
		Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner	
170 days	Control	31.6	28.9	47.4	21.1	39.5	39.5	42.1	34.2	35.5
	+ Biotin	38.5	23.1	48.8	23.1	51.3	20.5	38.5	33.3	34.6
First weaning	Control	66.7	66.7	75.0	69.4	83.3	33.3	83.3	41.7	54.9
	+ Biotin	68.8	65.6	62.5	46.9	81.3	34.4	71.9	25.0	57.0
Second weaning	Control	80.6	83.9	67.7	77.4	93.5	48.4	93.5	35.5	72.6
	+ Biotin	72.4	70.0	72.4	79.3	96.6	37.9	79.3	37.9	59.1
Third weaning	Control	88.9	96.3	81.5	92.6	92.6	51.9	92.6	44.4	80.1
	+ Biotin	63.6	72.7	81.2	81.2	86.4	45.5	100.0	40.9	71.6
Fourth weaning	Control	66.7	79.2	75.0	91.7	95.8	54.2	91.7	41.7	74.5
	+ Biotin	71.4	76.2	71.4	81.0	85.7	52.4	95.2	52.4	73.2

Effect of dietary biotin supplementation and age of sow on mean number of
lesions/claw

Period of examination	Treatment	Left Fore		Right Fore		Left Hind		Right Hind		Overall
		Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner	
170 days	Control	0.74	0.39	0.92	0.37	0.82	0.66	1.24	0.66	5.80
	+ Biotin	0.69	0.38	1.02	0.51	1.05	0.38	0.95	0.51	5.49
First weaning	Control	1.67	1.14	2.19	1.61	2.69	0.56	2.81	0.78	13.45
	+ Biotin	1.56	1.16	1.31	0.66	2.41	0.59	1.63	0.47	9.79
Second weaning	Control	2.55	1.81	1.42	1.52	3.06	0.84	3.26	0.55	15.01
	+ Biotin	1.69	1.55	1.31	1.48	3.03	0.48	2.76	0.66	12.96
Third weaning	Control	2.26	2.30	2.11	2.22	3.85	0.96	3.26	1.00	17.96
	+ Biotin	2.05	1.32	1.73	1.68	3.23	0.86	2.86	0.68	14.41
Fourth Weaning	Control	1.96	1.83	1.71	2.25	3.71	1.08	3.67	0.92	17.13
	+ Biotin	1.95	1.38	1.76	1.76	3.29	0.81	3.33	1.00	15.28

APPENDIX 9

Effect of dietary biotin supplementation and age of sow on the percentage
incidence of claws with defects for sows completing four parities

Period of examination	Treatment	Number of claws									Mean number of claws / sow with defects
		0	1	2	3	4	5	6	7	8	
170 days	Control (24)	29.2	33.3	25.0	4.2	4.2	0.0	4.2	0.0	0.0	1.4 ± 0.3
	+ Biotin (21)	19.1	28.6	33.3	9.5	4.8	4.8	0.0	0.0	0.0	1.7 ± 0.3
First weaning	C trol (24)	16.7	20.8	8.3	29.2	16.7	8.3	0.0	0.0	0.0	2.3 ± 0.4
	+ Biotin (21)	0.0	23.8	33.3	9.5	19.1	4.8	4.8	0.0	4.8	2.8 ± 0.4
Second weaning	Control (24)	16.7	12.5	12.5	33.3	8.3	12.5	0.0	4.2	0.0	2.6 ± 0.4
	+ Biotin (21)	14.3	19.1	9.5	14.3	14.3	28.6	0.0	0.0	0.0	2.8 ± 0.4
Third weaning	Control (24)	8.3	16.7	14.7	20.8	12.5	0.0	0.0	0.0	0.0	2.1 ± 0.2
	+ Biotin (21)	19.1	23.8	33.3	19.1	4.8	0.0	0.0	0.0	0.0	1.7 ± 0.3
Fourth weaning	Control (24)	16.7	29.2	20.8	25.0	8.3	0.0	0.0	0.0	0.0	1.8 ± 0.3
	+ Biotin (21)	28.6	4.8	38.1	19.1	4.8	4.8	0.0	0.0	0.0	1.8 ± 0.3

Note: Figures in parentheses indicate sample size.

13

APPENDIX 10

Effect of dietary biotin supplementation and age of sow on the percentage
incidence of claws with lesions for sows completing four parities

Period of examination	Treatment	Number of claws									Mean number of claws/sow with lesions
		0	1	2	3	4	5	6	7	8	
170 days	Control (24)	16.7	12.5	20.8	12.5	12.5	4.2	12.5	8.3	0.0	3.0 ± 0.5
	+ Biotin (21)	23.8	14.3	19.1	9.5	14.3	4.8	14.3	0.0	0.0	2.5 ± 0.5
First weaning	Control (24)	0.0	0.0	4.2	8.3	25.0	16.7	20.8	16.7	8.3	5.3 ± 0.3
	+ Biotin (21)	0.0	4.8	4.8	23.8	14.3	23.8	19.1	9.5	0.0	4.4 ± 0.4
Second weaning	Control (24)	0.0	0.0	4.2	4.2	16.7	16.7	16.7	25.0	16.7	5.8 ± 0.4
	+ Biotin (21)	0.0	9.5	4.8	9.5	14.3	9.5	14.3	14.3	23.8	5.3 ± 0.4
Third weaning	Control (24)	0.0	0.0	0.0	0.0	16.7	8.3	25.0	20.8	29.2	6.4 ± 0.3
	+ Biotin (21)	0.0	4.8	4.8	0.0	9.5	23.8	23.8	14.3	19.1	5.7 ± 0.4
Fourth weaning	Control (24)	0.0	0.0	0.0	4.2	16.7	8.3	25.0	16.7	29.2	6.2 ± 0.3
	+ Biotin (21)	0.0	0.0	0.0	4.8	14.3	28.6	19.1	9.5	23.8	5.9 ± 0.3

Note: Figures in parentheses indicate sample size.

14

Effect of dietary biotin supplementation and age of sow on mean number of lesions/claw for sows completing four parities

Period of examination	Treatment	Right Fore		Left Hind		Right Hind		Left Hind		Overall
		Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner	
170 days	Control (24)	0.75	0.42	0.88	0.29	0.92	0.83	1.25	0.88	6.21
	+ Biotin (21)	0.67	0.38	0.67	0.29	0.95	0.48	1.00	0.48	4.90
First weaning	Control (24)	1.42	1.25	2.13	1.83	2.58	0.54	2.63	0.83	13.21
	+ Biotin (21)	1.05	1.14	0.86	0.67	2.33	0.52	1.53	0.29	8.34
Second weaning	Control (24)	2.54	1.92	1.21	1.59	3.00	0.96	3.54	0.58	15.21
	+ Biotin (21)	1.67	1.81	1.29	1.67	2.81	0.43	2.81	0.67	13.10
Third weaning	Control (24)	2.21	2.29	2.17	2.33	3.92	0.96	3.38	1.08	18.33
	+ Biotin (21)	2.00	1.33	1.81	1.71	2.62	0.67	2.90	0.67	14.19
Fourth weaning	Control (24)	1.96	1.83	1.71	2.25	3.71	1.08	3.67	0.92	17.13
	+ Biotin (21)	1.95	1.38	1.76	1.76	3.29	0.81	3.33	1.00	15.28

Note: Figures in parentheses indicate sample size.

Theoretical inclusion levels for egg-white to bind all available biotin in diet in experiment 2

Total biotin in control diet   =   121.7 µg/kg
Available biotin in control diet =   31.5 ug/kg

It is estimated that 6 to 9 µg biotin is bound by 1 g egg-white. Assuming only 6 ug biotin is bound by 1 g of egg-white, then 46 g egg-white is needed to bind 32 µg biotin, giving an inclusion rate of 0.6%.

Actual inclusion rate of egg-white in diet A is 1%.

RECENT RESEARCH ON THE RESPONSES OF

POULTRY AND PIGS TO SUPPLEMENTARY BIOTIN

C. C. WHITEHEAD

P. H. BROOKS and P. H. SIMMINS

CONTENTS

RECENT FINDINGS ON THE EFFECT OF BIOTIN SUPPLEMENTATION ON
REPRODUCTIVE PERFORMANCE AND THE MAINTENANCE OF HOOF INTEGRITY
IN THE FEMALE PIG

P. H. BROOKS and P. H. SIMMINS

INTRODUCTION

The Roche symposium, London, 1977, included a report of a
trial conducted at Seale-Hayne College, in which supplementation
of breeding pig diets had improved hoof health in a herd
previously suffering from a high incidence of lameness (Brooks
1977). Supplementation of the diets of these pigs with
250 µg d-biotin/kg in pregnancy and 150 µg d-biotin/kg in
lactation for six months resulted in a 28% reduction in the
number of hoof lesions while control animals showed a 1%
increase.

In addition to the beneficial effects on hoof health, biotin
supplementation of die   also produced improvements in re-
productive performance. Most notably, weaning to remating
interval was reduced by 9.1 days (P < 0.05) and litter size
was increased by 1.2 pigs/litter in the case of second parity
sows and 0.4 pigs/litter in the third parity sows.

When this trial was initiated it was thought that biotin
deficiency was unlikely to occur spontaneously in pigs fed
conventional U.K. diets. Apart from a few isolated and
imperfectly controlled trials reported by Cunha (1972) there
was no experimental evidence to contradict the widely held
belief that the provision of biotin in normal diets, possibly

supplemented by biotin synthesized by the microbial popula-
tion of the lower gut, was sufficient to meet pigs' require-
ments.

However, in 1972, Yugoslavian workers reported that foot
lesions resulting from what they termed "concrete disease"
resolved within a matter of weeks when sows were fed very
high levels of biotin (Bujas et al 1972). Experimental
results from Switzerland (Glattli 1975) and field
observations in the U.K. (Hardy 1978 pers. comm.; Comben
1978) provided confirmation that at least some herd lame-
ness problems, having their genesis in foot lesions, were
biotin-related and could be resolved by high levels of
biotin supplementation. The time taken for resolution of
the problem was often not long enough for normal horn growth
and renewal to be the only factor in the recovery. Subject-
ive observations made by a number of authors suggested that
biotin might be exerting an influence either by supporting
more rapid scar tissue production or by increasing hoof
hardness and thereby affording greater protection to the
more sensitive underlying tissues of the hoof.

The occurrence of biotin responsive conditions in sow herds
initially produced more questions than answers. Were these
isolated incidents or merely the tip of the iceberg? Did
these cases imply that biotin was generally or specifically
undersupplied in diets or did the cases arise from the
chance presence of biotin antagonists? If biotin supply was
inadequate was this a new phenomenon or a long standing

problem that had previously been unrecognised? Finally and most important, what was the biotin requirement of the pig?

Although all these questions have not been answered, recent studies have considerably increased our understanding.

In an earlier review (Brooks 1978) it was demonstrated that changes which had occurred in the National Herd could, in theory, have led to a situation in which a previously adequate dietary provision might have been reduced to a sub-optimal or deficient level. Two factors in particular might have contributed to this situation. First, the average performance of breeding sows had increased and their food intake diminished over recent years (Fig 1). As a consequence, the amount of food consumed per weaner pig produced had diminished. Even if the composition of the diets fed had remained constant this would have meant that the biotin provision per kg piglet

CHANGE IN SOW PRODUCTIVITY AND FOOD PER WEANER
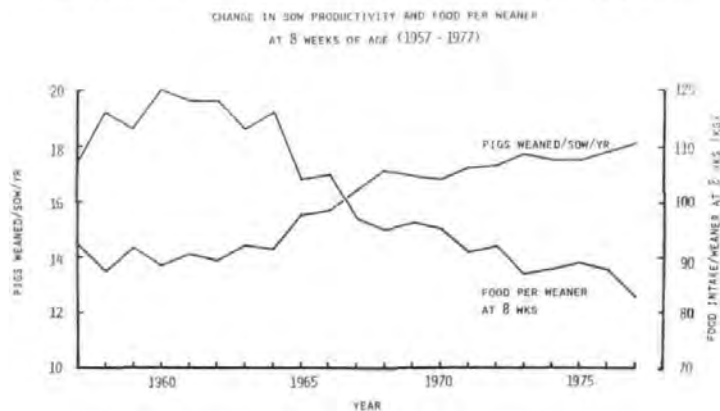AT 8 WEEKS OF AGE (1957 - 1977)

Figure 1.

produced would also have been reduced. However, the biotin content of diets had also been reduced due to changes in diet formulation resulting from changes in the relative price of raw materials on the U.K. market (Putnam 1977). The effect of these two concommitant changes was to reduce biotin content of the diet considerably but, more particularly, to reduce the available biotin content. As a consequence Brooks (1978) ca :ulated that the biotin provision per weaner at eight weeks probably dropped by about 27% between 1967 and 1976. During this same period the provision of vitamin A increased by 140% and vitamin D by 59%. (Figure 2)

CHANGE IN INTAKE OF VITAMINS A, D AND BIOTIN
PER WEANER AT 8 WKS EXPRESSED
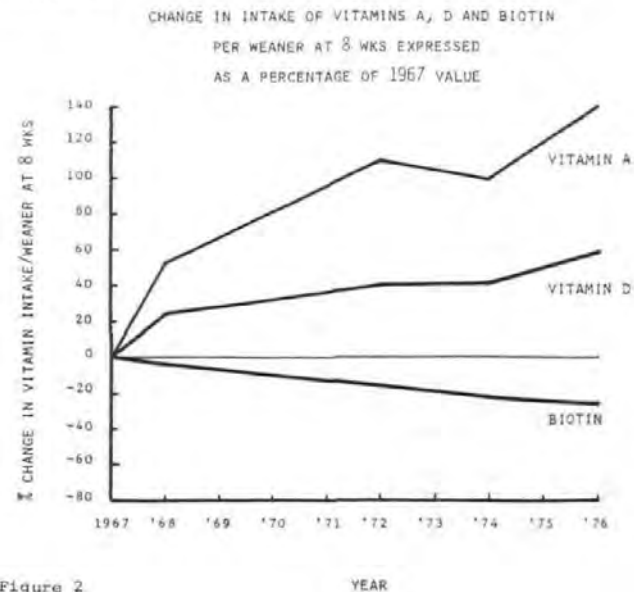AS A PERCENTAGE OF 1967 VALUE

Figure 2   YEAR

By extrapolating from Whitehead's estimated biotin requirements for poultry, Brooks (1978) calculated that the available biotin content of a sow diet would need to be between 137 and 535 µg/kg diet according to daily intake and sow liveweight (Table 1). If it was assumed that the daily intake increased in proportion to the sow's liveweight, an average value of

Table 1

Relationship between sow liveweight, daily feed intake, and dietary biotin level needed to meet suggested requirement of sow

Sow Liveweight (kg)	Suggested Available Biotin intake* (µg/day)	Dietary Requirement (µg/kg) to Meet Intake Requirement					
		Feed Intake (kg/day)					
		1.73	2.00	2.25	2.50	2.75	3.00
100	411	235	206	183	164	149	137
150	557	318	279	248	223	203	186
200	691	394	346	307	276	251	230
250	817	467	409	363	327	297	272
300	937	535	469	416	375	341	312

* Intake to provide 13 µg available biotin per kg metabolic body size ($W^{0.75}$).

300 µg/kg feed would meet this estimated requirement in the majority of cases. This figure is considerably in excess of the available biotin level likely to be found in a diet based on wheat, barley, white fish meal and extracted soya bean meal, which would be of the order 30-60 µg/kg.

Long-term trial

It appeared from these calculations that some sow diets might contain inadequate biotin. In order to test this hypothesis, a long term trial was initiated at Seale-Hayne College with the object of investigating the effect of biotin supplementation on the hoof health and reproductive performance of pigs which were clinically healthy at the commencement of the trial (Brooks and Simmins unpublished data).

Table 2

Composition of Diets	Diet "B" Pregnancy	Diet "A" Lactation
	%	%
Barley	50.0	50.0
Wheat	43.0	40.5
Fishmeal	5.0	7.5
Min/Vit	2.0	2.0
CP %	13.1	14.4
DE (MJ/kg)	13.1	13.1
Total Biotin µg/kg*	120	122
Available biotin µg/kg*	31	32

* Calculated values

In this trial, the treatments were imposed at 25 kg liveweight and continued for four parities. Two diets were used (Table 2). Diet A was fed during the growing period (until mating), during lactation and during the period from each weaning to first service. Diet B was fed during pregnancy. The supplemented group received the same diets with the addition of 350 µg d-biotin/kg (ROVIMIX H-2, Roche Products Limited). The animals were individually fed once a day; their feed allowance in pregnancy being determined by parity number and in lactation by litter size. The animals completed four parities unless culled prematurely.

Maintenance of Hoof ntegrity

Although the analysis of data has not been completed, prelim-
inary results indicate that supplementation did have a
significant effect on the maintenance of hoof integrity,
(Figure 3). Small but non-significant differences were
apparent by the time the gilts entered the herd (170 days of
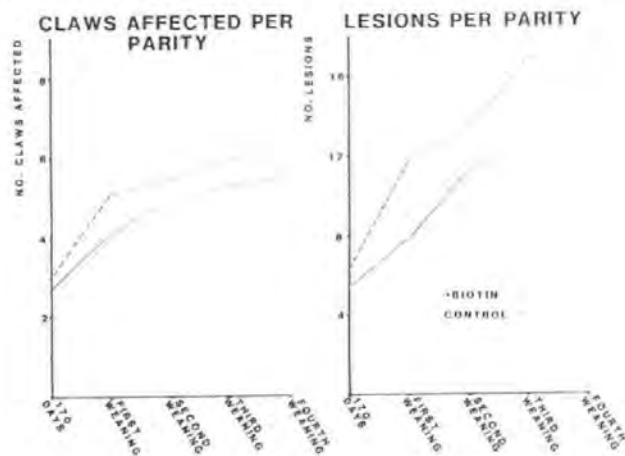age). By the end of the first lactation there were significant

Figure 3.

(P < 0.05) differences between the groups for numbers of
claws with lesions and number of lesions/pig. The differences
between the groups persisted up to the end of the fourth
parity.

This finding is of particular interest because it corrobor-
ates results obtained in two other recent trials. Triebel and

Lobsiger (1979) observed a substantial reduction in the
percentage of claws affected with cracks and with necrotic
or ulcerative lesions when gilt diets were supplemented with
biotin (Table 3). In their trial the basal diet was cal-
culated to have provided 55 μg available biotin/kg and the

Table 3

Effect of Biotin Supplementation on Claw Health of Gilts

Gilts	Biotin Added	Claw Horn Cracks		Necrosis and Ulcerative Lesions in the Claws	
		% of the affected claws		% of the affected claws	
		Medium + Severe	Total	Medium + Severe	Total
17	O	18.1	41.9	19.9	22.1
25	500 μg/kg for 4 months	3.1	7.5	2.0	2.5

(Triebel and Lobsiger 1979)

supplemented diets had an addition of 500 μg biotin/kg.

Most recently Penny *et al* (1980) reported that when gilts
were introduced as replacements into a herd and fed a biotin
supplement of 1160 μg/day in pregnancy and 2320 μg/day in
lactation they had significantly fewer white line lesions,
heel bruises and erosions than unsupplemented controls. The
severity score and number of lesions/pig were reduced by 17
and 18% respectively as a result of supplementation.

In their trial, replacement animals entering the herd with
minimal foot damage benefitted significantly from supple-
mentation. Older sows with a high initial incidence of

lesions did not show any improvement in the condition of their
feet even after 12 months supplementation.

The results obtained in these trials would indicate that, for
housed animals, a level of 56 µg available biotin/kg of diet
is certainly inadequate to support the growth and development
of the hoof and subsequently to maintain its integrity. It
would also appear that improvements in hoof condition
achieved by biotin supplementation in early life are maintain-
ed if the biotin supplementation is continued subsequently.

The effect of supplementation in older animals which have not
had the benefit of supplementation in early life depends on
the severity of the lesions present, the housing and flooring
conditions in the unit and the level of supplementation given.
Brooks et al (1977) obtained improvements in hoof condition
with supplements of 150-250 µg biotin/kg diet. Similarly
Grandhi and Strain (1980 personal communication) found that
supplementation of barley-wheat-soya diets with 200 µg biotin/
kg reduced the severity of lesions in both Lacombe and York-
shire sows housed in dirt lots during pregnancy and confine-
ment facilities in lactation. Despite these results, field
experience in the U.K. (Comben 1978) suggests that the much
higher levels suggested by Glättli (1975) of 2-3000 µg/kg are
often required to resolve established lesions.

Effect of Biotin on Hoof Hardness

The way in which biotin influences hoof hardness is still not
certain, but the most likely effect appears to be on the
physical characteristics of the hoof. In recent studies
(Simmins and Brooks 1980) attempts have been made to develop
quantitative techniques for measuring differences in hoof
hardness. It is possible to demonstrate differences in
compression strength between hooves (Table 4) and also
differences in hardness between different areas of the hoof

Table 4

Effect of Dietary Biotin intake on Compression Strength
of Growing Pig Hoof Material

	Biotin Intake of Pigs µg/kg $W^{0.75}$				
	0	2.6	15.6	81.6	SED
Compression Strength (MNm^{-2})	27.8	28.9	30.3	35.5	4.4
Modulus of Elasticity (MNm^{-2})	297	426	280	382	84

After Simmins & Brooks (1980)

Table 5

Effect of Dietary Biotin on Horn Strength as Estimated
using a "Durometer"

	Biotin Intake of Pigs µg/kg $W^{0.75}$				
	0	2.6	15.6	81.6	SED
Sidewall	56.5	59.4	59.4	58.5	1.6
Heel	8.9	4.6	6.4	8.6	1.2
Toe	39.0	43.8	43.0	41.8	2.9
Whiteline	32.8	34.8	33.0	36.3	1.7

Simmins & Brooks (1980)

(using a Durometer*) (Table 5) but the variability of readings

* (Shore Instrument Mfg. Inc.)

within treatment groups limits the confidence with which these findings can be interpreted. Nevertheless, future development of these techniques may lead to a greater understanding of the role played by biotin in the maintenance of hoof integrity.

Effects of Biotin on Reproduction

In early studies, an unexpected result of supplementing sow diets with biotin was the improvement of a number of parameters of reproductive performance (Brooks *et al* 1977). This improvement was unrelated to any effect on lameness, that is, it did not result from increased mobility of the sow or improvements in the sow's willingness to mate. Although improvements in reproduction were unexpected they should not occasion incredulity. The involvement of biotin in a considerable number of metabolic pathways makes it inevitable that a whole range of body functions would be impaired if the biotin provision is inadequate.

Some recent studies confirm that biotin has an influence on reproduction in the pig. Halama (1979) has published results obtained from a 170 sow unit in Austria. The sows in this herd were fed diets which, it was calculated, would provide 145-220 µg available biotin/day in pregnancy and 300-450 µg available biotin/day in lactation. Biotin supplementation was introduced due to the poor skin condition of sows in the herd and the high incidence of lameness. Biotin intake was increased to 330-500 µg/day in pregnancy and 750-1170 µg/day in lactation. In addition to producing marked improvements

in both skin condition and hoof health, supplementation produced a reduction of 2-3 days in the weaning to remating interval, an increase in conception rate from 76 to 85% and a reduction in the incidence of small litters (8 pigs or less). Völker & Smith (1980) have reported improvements in reproductive performance in two other trials. In a large Spanish study, involving in excess of 100 sows per treatment, supplementation resulted in a 2.9% increase in the number of pigs reared and a 9.3% reduction in stillborn pigs. In a second trial (Spencer cited by Völker & Smith 1980) sows whose diets were supplemented with biotin produced 0.42 more piglets per sow per litter and had a shorter inter-farrowing period (3.6 days). Unfortunately the levels of supplementation used in these two trials were not given.

Grandhi and Strain (1980 pers. comm.) also looked at reproductive performance in their trial. Although foot lesions were influenced by supplementation, reproductive function was not significantly affected.

In the long term trial recently completed at Seale-Hayne College, (Brooks & Simmins 1980 unpublished data), supplementing sow diets with 350 µg/kg d-biotin also enhanced reproductive performance. As the statistical analysis of all the data has not been completed the significance of some of the results must be viewed with caution. Nonetheless the trends observed show conclusively the benefits of supplementation. Over four parities, supplemented sows farrowed 0.1 additional pigs/litter and weaned 0.3 additional

pigs/litter (an increase of 3.4%) (Fig. 4).

It is interesting to note that the number of pigs born/litter
in the control group was actually lower at second farrowing
than at the first. This may result from the first lactation
placing a proportionally greater demand on the unsupplemented
animals. This hypothesis appears to be supported by the
results of plasma biotin assays on samples from late
pregnant and newly weaned sows. (Table 6)

Table 6

Biotin Levels in Plasma - (ng/100 ml)

	Pre-Farrow	Weaning
Control	111	103
Supplemented	192	196

Supplementation also affected litter weight at birth and at
weaning. At weaning, litters from supplemented sows averaged
2.6 kg/litter (3.5%) more than those from control sows.

The most spectacular improvement produced by supplementation
was in breeding frequency. The intervals from weaning to
oestrus, and weaning to effective service were improved in
each parity (Fig. 5). Pooled data from the first three
weanings indicated that supplemented sows returned to
oestrus 2.9 days sooner than controls and conceived 6.1 days
sooner (P < 0.05).

Post weaning performance of the two groups actually differed
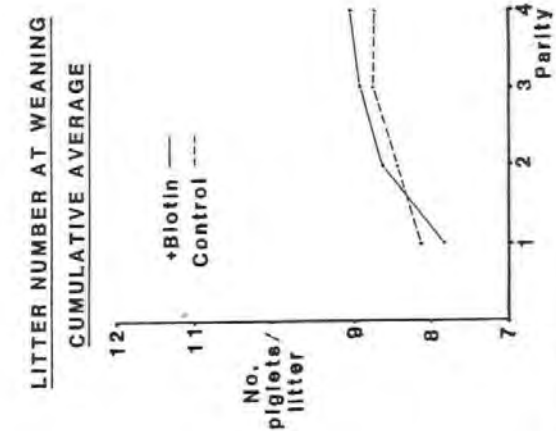more than these figures suggest. Within the herd it was

LITTER NUMBER AT WEANING
CUMULATIVE AVERAGE

Figure 4b.

LITTER NUMBER AT BIRTH
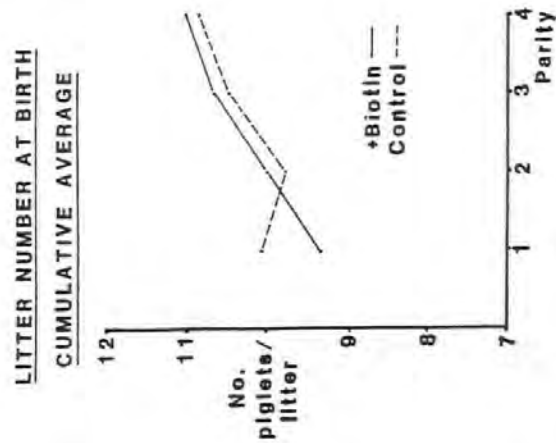CUMULATIVE AVERAGE

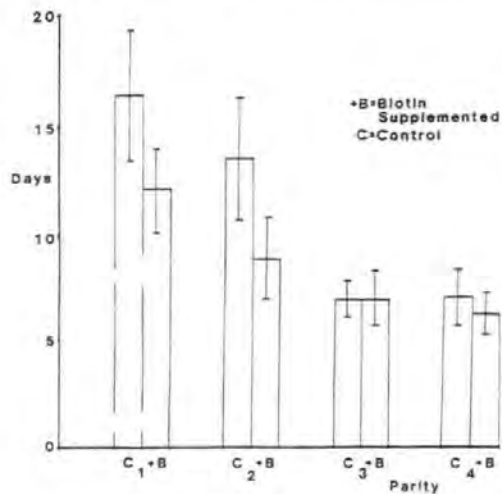Figure 4a.

WEANING TO OESTRUS INTERVAL

Figure 5.

policy to attempt to induce oestrus in sows which did not
show oestrus within 29 days of weaning by injecting them with
PG 600 (Intervet Limited). Fifteen control sows were treated
in this way of which eleven subsequently became pregnant,
compared with six supplemented sows of which four conceived.

Of the sows returning to oestrus within 29 days of weaning,
conception rate was 95% for controls and 100% for supplemented
animals.

An attempt has been made to assess the impact of supplementa-
tion on herd productivity (Fig 6). A figure which is

WEANING TO EFFECTIVE SERVICE INTERVAL

Figure 6.

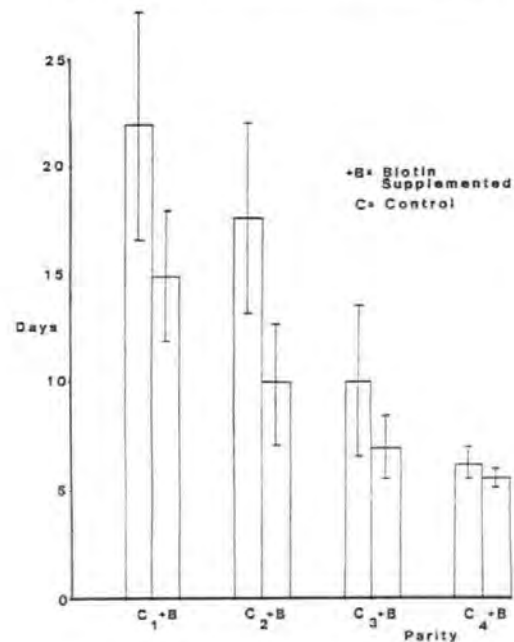equivalent to the rate of piglet production per sow/year can
be calculated from the appropriate litter performance and
service interval data for each treatment. The control sows
had a higher production rate in their first parity, but there-
after the supplemented sows were superior. As a result, the
difference in favour of the supplemented sows increased with
each additional parity. By the fourth parity the cumulative

production was equivalent to 18.7 pigs/sow/year for the
control sows and 20.5 pigs/sow/year for the supplemented
animals. Thus for a herd with the age profile achieved in
this experiment supplementation produced a response equivalent
to 1.8 piglets/sow/year. (Figure 7)
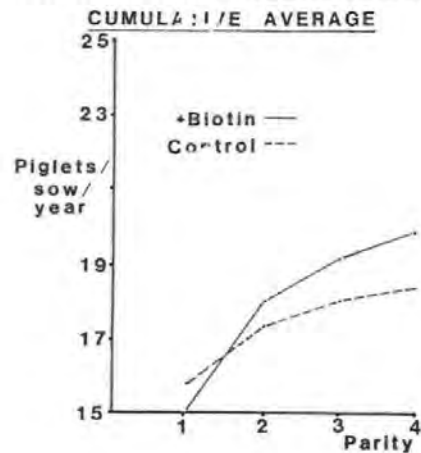
PIGLET PRODUCTION AT WEANING

CUMULA:I/E AVERAGE

Figure 7

Conclusions

From the foregoing discussion it must be concluded that the
modern, highly productive sow may not always receive
sufficient dietary biotin for the growth of horn tissue,
for the maintenance of hoof integrity or to support optimum
reproductive performance. What has not yet been clearly
established is the dietary levels of the vitamin needed to
eliminate these problems. A specific metabolic criterion
for the assessment of biotin status would be extremely
valuable in determining requirements. The possibility of
using pyruvate carboxylase has been investigated (Simmins 1979
unpublished data; Whitehead et al 1980 and Glatzle 1979).
Although blood pyruvate carboxylase activity was related to
biotin status, the activity level was more than 100 times
lower than in the chicken. Such a low level of activity
limits its suitability as a measure of biotin status in the
pig. Plasma biotin levels can be useful in research when
the experimental technique limits variability but, as a
measure of adequacy in the field, they lack precision and
can easily be misinterpreted. Samples from a number of
animals are required before reliable conclusions can be
drawn.

Studies currently being undertaken at Seale-Hayne College
suggest that biochemical changes in depot fat may provide a
means of assessing the biotin status of the animal. If
biotin dependent changes in fat biochemistry can be demon-
strated to be both characteristic and repeatable they could

better reflect the animals medium to long term biotin status.

In the absence of definitive determinations any statement of requirement must be speculative. From the work of Halama (1979) it can be demonstrated that a level of 75 µg available biotin/kg diet is certainly insufficient. From the work at Seale-Hayne the producer could deduce that a supplementation level of 350 µg/kg offered a very significant cost benefit. It can be calculated that in that trial the additional margin produced by an extra 1.8 pigs/year was at least fifteen times the cost of the vitamin supplement. However, work in progress at present suggests that for the rearing gilt and the lactating sow the optimum level of biotin may be in excess of 350 µg/kg diet. It is to be hoped that in the next few years the techniques which are currently being developed come to fruition and provide the means whereby the biotin requirements of the pig can be precisely and confidently established.

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SUMMARY

In 1977 Brooks, Smith and Irwin published the results of a trial in which sows suspected of having developed a spontaneous deficiency of biotin were given dietary supplements of pure d-biotin (ROVIMIX H). As a result of biotin supplementation the hoof integrity of the suspectedly deficient sows improved. Surprisingly there were also improvements in the

reproductive performance of the supplemented animals, in
particular an improvement in litter size in second parity sows
and a reduction in the weaning to remating interval.

As a result of these findings a long term trial involving
eighty females was initiated to investigate whether biotin
supplementation of clinically healthy female pigs affected
reproductive performance and/or the maintenance of hoof
integrity. Female pigs were fed diets based on barley,
wheat and fishmeal, which have been shown to have low
available biotin status, calculated to provide 31 µg available
biotin/kg. Diets for experimental group were supplemented
with 350 µg/kg d-biotin. The animals were allocated to
treatment at 25 kg liveweight and remained on treatment for
four parities. Hoof lesions were recorded at 170 days of age
and at each successive weaning, blood plasma samples, taken
two days prior to parturition and at weaning, were assayed
for biotin and detailed performance records were maintained
throughout the trial.

Data was presented which indicated that a number of
parameters of reproductive performance are improved by
supplementing diets with biotin and that the incidence of
hoof lesions and the categories of lesion recorded are also
influenced by the animals biotin status. The economic
significance of these results was also discussed.

WINTER MEETING 1980

The effect of dietary biotin level on the physical

characteristics of pig hoof tissue.

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Recent studies at Seale-Hayne College have confirmed that the dietary intake of available biotin plays an important part in the maintenance of hoof integrity in the pig.   However, the mode of action of biotin in this context has not yet been elucidated.   Several workers have made the subjective observation that pigs on low biotin or biotin deficient diets have unusually soft horn compared with pigs receiving biotin supplemented diets.   Data which has accumulated in the course of a long term trial suggest that differences in the rate of development and type of foot lesion categorized in supplemented and unsupplemented animals might result from biotin altering the physical characteristics of the horn produced and hence affecting its durability and ability to withstand traumatic injury.

The object of the trial reported was to assess the possibility of developing an objective measure of hoof strength and to investigate whether various physical characteristics of the hoof were influenced by dietary biotin intake.

Four replicates each of eight gilts were randomly allocated, at 25 kg. liveweight to one of four dietary treatments.

A.   Basal diet + 5% dried egg white.
B.   Basal diet.
C.   Basal diet + 120 mcg/kg d.biotin.
D.   Basal diet + 720 mcg/kg d.biotin.

The basal diet was formulated from commonly used raw materials which have been demonstrated to have a low available biotin content (Table 1).

Table 1.

PERCENTAGE COMPOSITION OF CONTROL DIET AND BIOTIN AVAILABILITY

	Diet	Assumed Biotin Level(mcg/kg)	Total Biotin (mcg/kg)	Assumed Availability	Available Biotin mcg/kg
Barley	26.26	150	39	30	11.7
Wheat	48.45	100	48	10	4.8
Wheatfeed	13.19	310	40	10	4.0
Fishmeal	10.10	110	11	100	11.0
Min/Vit	2.0				
				TOTAL	40.5

Treatment A was a negative control diet.   The egg white supplied avidin calculated to be sufficient to inactivate the biotin present in the basal diet.

The gilts were individually fed, once daily, on a scale related to metabolic body weight.   Gilts on treatments B, C & D were fed at the rate of 110g diet/kg$W^{0.75}$.   Gilts on treatment A were fed to a 5% higher scale to ensure that the intake of other nutrients was at least equal to that on the other treatments.

The gilts were slaughtered on reading 86kg liveweight.   The hooves were severed from the carcass above the coronet prior to scalding and retained for subsequent analysis.

Using an Instron Materials Testing Instrument tests were conducted on samples from predetermined areas of the horn.   The tests performed were:-

1.   Puncture Test.

Samples of horn were dissected from underlying tissues at two predetermined areas:-
Area A on the abaxial sidewall at a point halfway from the leading edge to the rear edge on the horizontal axis and two-thirds of the distance from the coronet to the volar surface on the vertical axis.   Area B. on the volar surface immediately behind the white line.   The samples were pierced using a 3mm probe.

2. Compression Test

Rectangular samples 3mm in length and 3mm broad were cut from the sidewall on the leading edge of the right front outer hoof. The samples were compressed in a plane perpendicular to the orientation of the horn tubules until the sample sheared (yield point). Values for compression strength and modulus of elasticity were calculated.

Results of the trial are summarized in Table 2.

Table 2.

PERFORMANCE DATA AND PHYSICAL MEASUREMENTS OF HOOF

Treatment	A	B	C	D	SED
D.L.W.G.(g/day)	764	720	696	706	26
F.C.R.	2.83	2.81	2.88	2.86	0.12
Puncture test (Area A)(N)	327	335	325	317	25
(Area B)(N)	326	408	337	374	31
Compression (MNm^{-2})	27.8	28.9	30.3	35.5	4.4
Modulus of elasticity (MNm^{-2})	297	426	280	382	84

Neither daily liveweight gain nor food conversion ratio were significantly affected by dietary biotin level. The slightly higher growth rate in Treatment A may be explained by the somewhat higher energy and crude protein intake provided by the scale of feeding on this treatment.

There were no significant treatment differences in the values for the physical tests performed. However, compression strength did increase with the level of dietary biotin.

The suitability of the puncture test in assessing this type of material is in some doubt as the profile of the sample produced introduces considerable error into the measurement.

The compression test appears to be worthy of further investigation and development. However, in young pigs in particular the limited size of sample which can be prepared continues to present the greatest problem in developing the technique.

The effect of dietary biotin supplementation on lipid
fractions of sow milk fat in early and late lactation.

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Recent studies at Seale-Hayne College have confirmed that the dietary intake of available biotin
plays an important part in the reproductive performance of the sow. Pooled data for the first three
weanings in a long-term trial have indicated that the biotin supplemented sows returned to oestrus
2.9 days sooner than control animals and conceived 6.1 days earlier (p < 0.05).

However, deciding whether the biotin status of a commercial animal is a constraint on its
performance is still a problem. The techniques for the analysis of the biotin status of the pig must
be treated with caution. The most commonly used measure is plasma biotin level, however this is only
suitable as an indicator of the biotin status of groups of animals. Plasma biotin determinations from
suspectedly deficient sows have indicated that biotin levels were higher in the pregnant animal than
the lactating sow suggesting that the extent of deficiency may vary with reproductive status. Biotin
demand is likely to be at its greatest during lactation for two reasons. First, biotin is secreted in
the milk and this in turn increases the dietary biotin requirement. Secondly, lactating sows usually
catabolise fat. As there is a biotin requirement for fat catabolism, a marginal biotin supply may
directly influence milk production. Milk fat composition reflects the changes in composition of plasma
triglycerides and so may respond rapidly to changes in the biotin status of the sow. Subcutaneous
depot fat responds to long-term changes in biotin in nutrition and may not reflect transient changes in
the biotin status of the animal.

Evidence has accumulated at Seale-Hayne College that a first stage fractionation of the tissue
lipids into phospholipids and neutral lipids produces results which show a biochemical response to
marginal differences in biotin supply. The object of this trial was to determine whether biotin
supplementation of diets formulated from commonly used ingredients and fed according to current commercial
practice, would influence the fatty acid profile of the phospholipid and neutral lipid fractions of milk
fat in early and late lactation.

Two groups of 9 and 6 sows in either their third or fourth lactation were fed either:

i. a negative control diet calculated to provide 36 mcg available biotin/kg or
ii. a control diet plus supplementary biotin at 350 mcg biotin/kg for a minimum of eighteen months.

The basal diet was formulated from commonly used raw materials which have been demonstrated to have a
low available biotin content (Table 1).

Table 1

PERCENTAGE COMPOSITION OF CONTROL DIET DURING LACTATION AND BIOTIN AVAILABILITY

	Diet	Assumed Biotin level (mcg/kg)	Total Biotin (mcg/kg)	Assumed (%) Availability	Available Biotin (mcg/kg)
Barley	50.0	150	75	30	22.5
Wheat	40.5	100	40.5	10	4.1
Fishmeal	7.5	110	8.3	30	9.1
Min/Vit	2.0				

Shoulder fat and milk samples were taken at two days following parturition and milk samples at weaning
(35 days). Extraction of fat from the milk was undertaken by the Rose-Gottlieb method. Methyl esters
of the phospholipids and neutral lipids of milk and shoulder fat were produced by established techniques.
Four fatty acids were studied, palmitic acid (C 16:0), palmitoleic acid (C 16:1), stearic acid (C 18:0)
and oleic acid (C 18:1) and the ratio of the saturated to unsaturated fatty acids examined. The results
are given in Tables 2, 3 and 4.

Table 2

SOW MILK PHOSPHOLIPIDS

Fatty Acid	Early Lactation Control (9)	+Biotin (6)	SED	Late Lactation Control (5)	+Biotin (4)	SED
18:1	30.4	30.8	5.1	34.3	35.9	6.4
18:0	5.5	4.4	1.3	4.3	3.4	1.5
16:1	13.4	14.1	1.8	14.1	12.7	3.2
16:0	50.8	50.7	4.7	47.2	45.7	6.3
18.1+16:1	43.8	44.8	5.1	48.5	51.0	5.3
18:0/16:0	56.2	55.2	5.3	51.5	49.0	5.3

Table 3

SOW MILK NEUTRAL LIPIDS

	Early Lactation			Late Lactation		
Fatty Acid	Control (9)	+Biotin (6)	SED	Control (5)	+Biotin (4)	SED
18:1	37.9	37.6	4.8	39.2	48.2	6.8
18:0	2.7	1.9	1.2	2.4	5.1	1.3
16:1	16.3	18.5	2.2	15.9	10.9	2.7
16.0	43.3	42.5(a)	3.5	42.1	35.9(a)	5.4
18:1+16:1	54.2	56.0	3.6	55.1	59.2	5.1
18:0/16:0	45.8	44.0	3.6	45.2	40.9	5.0

(a) means with the same superscript differ at P < 0.05

Table 4

SOW SHOULDER FAT EARLY LACTATION

	Phospholipid			Neutral Lipid		
Fatty Acid	Control (9)	+Biotin (5)	SED	Control (9)	+Biotin (5)	SED
18:1	44.6	43.6	4.5	60.9	62.5	3.7
18:0	19.1	23.6	3.4	7.6	8.1	1.4
16:1	4.2	4.6	2.2	5.2	4.2	1.6
16:0	29.9	28.5	2.4	26.5	25.2	3.6
18:1/16:1	51.0	48.0	4.3	66.2	66.7	3.9
18:0/16:0	49.1	52.1	4.3	33.9	33.3	3.8

Note: figures in parentheses indicate size of sample.

The phospholipid fraction was more unsaturated than the neutral lipids in both early and late lactation for the milk fatty acids and in early lactation for the shoulder fat samples. The proportion of palmitoleic acid was higher in milk fat compared to the shoulder fat due to a desaturase system in the milk fat. There was no significant difference between the ratio of unsaturation to saturation in the milk fatty acids in early and late lactation for biotin supplemented and unsupplemented sows. However, the level of unsaturation did increase for both lipid fractions and the proportion of palmitic acid decreased significantly (p < 0.05) for the neutral lipids in the supplemented group.

The limited scale of the current trial makes it unwise to attempt to draw definitive conclusions from the data. Nevertheless, the treatment differences observed, though not statistically significant do indicate the need for further more detailed studies of the effect of dietary biotin intake on depot and milk fat composition.

The Effect of Supplementing Breeding Pig Diets with Biotin on the Maintenance of Hoof Integrity

P. H. Brooks and P. H. Simmins

The Effect of Supplementing Breeding Pig Diets with Biotin on the Maintenance of Hoof Integrity

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Abstract

Eight crossbred female pigs were fed, from 25 kg either basal cereal/fishmeal diets containing 31 mcg available biotin/kg or basal diets supplemented with 350 mcg d-biotin/kg. Supplemented females had fewer claws affected with lesions and less hoof lesions/sow at 170 days of age and after weaning each of their first 4 litters. The differences were significant following first weaning ($p < 0.05$). In a second trial compression strength of hoof horn increased with dietary biotin level over the range 0–720 mcg/kg. The results confirm that commercial diets may be formulated which provide inadequate biotin to maintain hoof integrity.

Introduction

It has long been established that pigs with induced biotin deficiency develop hoof lesions. It is widley believed that pigs fed practical diets on commercial units obtain their total requirement for biotin from their food ingredients. Recently, evidence has been presented which indicates that the supply of biologically available biotin may be sub-optional in commercial diets for sows and that clinical symptoms similar to those reported for pigs with induced deficiency may be responsive to biotin supplementation.

In trials at Seale-Hayne College (2) the incidence of hoof lesions in sows was reduced by 28% when the pregnancy and lactation diets were supplemented with respectively 250 and 150 mcg, d-biotin/kg (ROVIMIX H; Roche Products Ltd.) for six months. Work in Yugoslavia (1) indicated that established lesions may resolve in 1–2 months if the diets of sows are supplemented with much higher levels of biotin (2000 mcg/kg). This finding has been confirmed in field cases in the U.K. (3).

This report presents interim results from two trials conducted to study the effect of biotin supplementation on the maintenance of hoof integrity in clinically normal pigs.

1

Materials and Methods

Trial 1: Eighty crossbred female pigs were allocated at 25 kg liveweight to one of two dietary treatments which were continued through four parities. The control group received pregnancy and lactation diets based on barley wheat and fishmeal and containing respectively 13.1% and 14.4% crude protein. The diets were calculated to provide 31 mcg/kg available biotin.

The supplemented sows received the same diets with 350 mcg/kg biotin added. All females were individually fed once daily throughout the trial.

The feet of the pigs were individually examined at 170 days of age and at successive weanings. Detailed records were made of location and type of lesion present in the hoof.

Trial 2: Thirty-two gilts were allocated at 25 kg to one of four dietary treatments.
A. Basal diet +5% dried egg white (calculated to provide sufficient avidin to inactivate natural biotin).
B. Basal diet (calculated to supply 41 mcg available biotin/kg).
C. Basal diet +120 mcg/kg d-biotin.
D. Basal diet +720 mcg/kg d-biotin.

Pigs in treatments B, C and D were fed at the rate of 110 g diet/kg W 0.75/day. Treatment A was fed to a 5% higher scale. Pigs were slaughtered at 85 kg liveweight and hoof horn subjected to physical analyses.

Results and Discussion

Treatment effects on the number of claws with lesions and mean number of lesions per sow are shown in Table 1.

Treatment differences were small at 170 days of age but increased considerably during the first parity. The integrity of the sows hooves continued to deteriorate throughout the trial but to a lesser extent in the case of the supplemented animals. This result suggests that a high biotin status early in the pigs breeding life confers some advantage to the hoof tissue and reduces the rate at which lesions develop. This finding is in line with the recent report of PENNY et al. (5) who supplemented diets for gilts with 1160 and 2320 mcg in pregnancy and lactation respectively and found significant treatment differences in number and severity of lesions and for five different categories of lesion. The mechanism by which biotin protects the hoof from damage is not known. Lesions present differently according to the flooring and management system provided for the animal. A noticeable difference in hoof hardness in deficient animals has been reported (2, 3, 5) and this may render the hoof more liable to wear and/or traumatic injury. In the second trial (Table 2) there were no significant differences in the values for the physical tests performed. However, compression strength increased with the level of dietary biotin. The difficulty in preparing hoof material from young pigs for such tests may well

Table 1. *Effect of biotin supplementation on the incidence of hoof lesion*

Examination	No. sows Control+Biotin		Mean No. claws with lesions Control+Biotin		Mean No. lesions per sow Control+Biotin	
170 days	38	39	3.0	3.7	6.3	5.5
1st weaning	36	32	5.1[a]	4.1[a]	11.9	7.9
2nd weaning	31	28	5.5	5.0	13.3	11.3
3rd weaning	27	23	6.0	5.3	17.0	12.5
4th weaning	24	21	5.8	5.6	14.8	13.9

[a] Means differ significantly ($P < 0.05$)

Table 2. *Effect of dietary biotin on performance and hoof characteristics*

Treatment	A	B	C	D	SED
D.L.W.G. (g/day)	764	720	696	706	26
F.C.R.	2.83	2.81	2.88	2.86	0.12
Puncture test					
(Sidewall) (N)	327	335	325	317	25
(Toe) (N)	326	408	337	374	31
Compression (MNm^{-2})	27.8	28.9	30.3	35.5	4.4
Modules of elasticity (MN^{-2})	297	426	280	382	84

obscure differences which though small are of significance over the lifespan of the breeding animal.

The results of these recent studies indicate that biotin plays a part in the maintenance of hoof integrity and confirm that commercial diets not supplemented with the vitamin may contribute to the incidence of foot lesions and lameness commonly encountered on pig units.

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Supplementary biotin for sows: Effect on reproductive characteristics

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Eighty female pigs were fed from 25 kg liveweight either basal diets calculated to provide 32 μg available biotin/kg (control diet) or basal diets supplemented with 350 μg biotin/kg. Reproductive performance was studied over four parities. Sows receiving supplementary biotin returned to oestrus 2·9 ± 1·7 and conceived 6·1 ± 1·4 days sooner than controls (P < 0·05). Of those sows receiving supplementary biotin, more returned to oestrus and conceived within 10 days of weaning (83·2 per cent v 74·6 per cent and 80·6 per cent v 71·8 per cent respectively) and fewer were treated for anoestrus (7·3 per cent v 17·0 per cent) than those on the control diet. Supplementing diets increased the annual productivity of sows completing four parities by 1·42 ± 1·02 pigs/sow/year (P > 0·05) and increased the total weight of weaner produced/sow/year by 17·3 ± 7·4 kg (P < 0·05). It was concluded that the majority of commercial dietary formulations would require supplementation with biotin in order that sows may express their full reproductive potential.

BIOTIN, a water soluble B group vitamin, has a number of important biochemical functions. It is a cofactor in a number of enzyme systems involved with carboxylation and transcarboxylation reactions and consequently has a significant effect on carbohydrate metabolism, fatty acid synthesis, amino acid deamination, purine synthesis and nucleic acid metabolism (Lynen 1967). Consequently, an inadequate supply of the vitamin results in clinical signs including alopecia, dermatitis and the development of claw lesions (Tagwerker 1974, Glättli and others 1975).

For many years after biotin was identified as an essential nutrient for the pig it was believed that the amount of biotin provided by normal dietary formulations, possibly supplemented by biotin produced by microbial synthesis in the lower gut, was adequate to meet the requirements of the animal. However, Brooks and others (1977) noted clinical signs in a commercial herd which corresponded closely with those reported for animals with induced biotin deficiency. Dietary supplementation of these animals with biotin reduced the incidence of claw lesions. Furthermore, supplementation produced a significant increase in the litter size of second litter sows and a significant reduction in the weaning to remating interval. In subsequent trials supplementation of basal diets calculated to provide between 29 and 155 μg available biotin/kg with 100 to 2000 μg biotin/kg has resulted in improvements in litter size ranging from 4 to 14 per cent (Halama 1979, Easter and others 1979, Pederson and Udesen 1980, Michel and Mastachi 1981, Penny and others 1981, Robres Serrano and Garcia de la Calera 1981). The interval between weaning and remating was reduced by 13 per cent in a trial by Bryant and others (1981) and by 18 per cent in that of Pederson and Udesen (1980). In only two trials (Newman and Elliot 1980, Grandhi and Strain 1981) did supplementation fail to produce an improvement in reproductive characteristics.

The trials noted above were all of relatively short duration involving either first and second parity animals only or animals of mixed age studied over one or two reproductive cycles.

The trial reported here was undertaken to investigate the long term effect of dietary biotin supply on the productivity of female pigs which were clinically healthy at the start of the trial period. In order to do this replacement gilts were allocated to treatment at 25 kg liveweight and remained on their respective treatment for four successive parities.

Materials and methods

Eighty gilts, comprising 40 pairs of full sibs, out of either landrace cross large white or large white sows mated to landrace boars were allocated to one of two dietary treatments at 25 kg liveweight. One gilt from each sib pair was fed on basal control diets, the other gilt received the same diets supplemented with 350 μg biotin/kg (Rovimix H2; Roche). The sows remained on their respective treatments until their fourth litters were weaned.

The composition and calculated analysis of the two basal diets (A and B) is given in Table 1. The raw materials selected had been shown previously to have a low available biotin content for the chicken (Anderson and Warnick 1970, Frigg 1976). In order to minimise degenerative interactions between micronutrients, the vitamins, minerals and the choline chloride were prepared in separate premixes. They were combined into a single premix immediately before incorporation into the diets which were mixed on a regular basis (usually weekly).

On allocation to treatment the gilts were fed 1·1 kg diet B/day. In each of the following two weeks the daily allocation was increased by 100 g/day and in subsequent weeks by 200 g/day to a maximum of 3 kg/pig/day. This level was maintained until 24 hours after mating. In parities one to four

TABLE 1: Composition and calculated analysis of diets

	Diet A	Diet B
Composition %		
Barley	50·0	50·0
Wheat	43·0	40·5
Fishmeal	5·0	7·5
Mineral/vitamin supplement*	2·0	2·0
Calculated analysis %		
Oil	2·1	2·1
Crude protein	13·1	14·4
Crude fibre	3·6	3·5
Total lysine	0·58	0·69
Methionine and cystine	0·47	0·52
Threonine	0·42	0·48
Calcium	0·92	1·07
Phosphorus	0·57	0·64
Salt (NaCl)	0·48	0·51
Digestible energy (MJ/kg)	13·15	13·11
Total biotin (μg/kg)	120·2	121·7
Available biotin (μg/kg)	31·1	31·5

*Mineral/vitamin supplement provided per kg diet: vit A 15,000 iu; vit D3 2000 iu; vit E 20 iu; vit K 4 mg; vit B1 2 mg; vit B2 5 mg; vit B6 4 mg; vit B12 15 mg; nicotinic acid 18 mg; pantothenic acid 15 mg; folic acid 1 mg; choline 300 mg; iron 100 ppm; cobalt 1·5 ppm; manganese 50 ppm; copper 10 ppm; zinc 80 ppm; iodine 3 ppm; selenium 0·1 ppm. Plus calcium, phosphorus and salt to give analysis above

TABLE 2: Total biotin levels in feed

Description of feed	Calculated level*	Assay results	
		Mean	Range
	(μg/kg)	(μg/kg)	(μg/kg)
Wheat	100	90	76–113
Barley	140	129	89–236
White fish meal	200	126	70–227
Diet A (no added biotin)	122	116	88–153
Diet A (plus biotin)	472	430	356–484
Diet B (no added biotin)	120	104	88–172
Diet B (plus biotin)	470	427	372–486

*Calculated from published values

TABLE 3: Biotin levels in plasma

	Control (ng/100 ml)	Supplemented (ng/100 ml)	se_D
Prefarrowing			
Mean	111	192	24·8**
Range	43–254	65–303	
	(n = 15)	(n = 13)	
Weaning			
Mean	103	196	31·3**
Range	39–307	92–546	
	(n = 19)	(n = 15)	

**Difference significant at P < 0·01

the pregnancy feed allowance was respectively 1·8, 2·0, 2·1 and 2·2 kg diet A/day. Diet B was fed in each lactation. The daily allowance increased from 2 kg on day 1 by 400 g/day increments to a maximum of 2 kg + 400 g/piglet suckled. Allocations were adjusted in the case of subsequent piglet deaths. From weaning until 24 hours after mating sows received 3·5 kg diet B/day. The sows were fed once daily throughout the trial and water was freely available at all times.

Piglets had free access to a proprietary 'creep' feed from seven days of age.

From allocation to treatment at 25 kg until 170 days of age the gilts were reared in isolation from mature animals. At 170 days of age they were moved to sow yards and housed adjacent to sexually mature boars. All unmated gilts, and subsequently all weaned sows, were tested for oestrus daily using boars. Gilts were mated at their first post pubertal oestrus and sows at their first post weaning oestrus. Two supervised matings were given whenever possible. Sows failing to exhibit oestrus by 29 days post weaning were treated with serum and chorionic gonadotrophin (PG 600; Intervet). Sows failing to exhibit oestrus within seven days of such treatment were slaughtered.

The sows were housed in buildings with solid concrete floors and some straw bedding throughout the trial. In pregnancy they were group housed in sow yards with a covered lying area. Sows farrowed in crates and were moved to individual 'follow-on' pens after seven days. They remained in these pens until weaning which took place on the first Thursday after the sows reached 35 days lactation. At weaning the sows returned to the sow yards.

Comprehensive records of sow and litter performance were maintained and sow weight change was also recorded.

Blood samples were taken for the assessment of plasma biotin from a representative sample of sows in each parity. Samples were taken within two days of farrowing and at weaning. Blood samples (10 ml) were obtained from the caudal vein, heparinised and centrifuged at 3000 rpm. The plasma was pipetted off, deep frozen and assayed for biotin using the method of Frigg and Brubacher (1976). Samples of raw materials and complete diets were also analysed using the same method.

Data were analysed within parities using a one way analysis of variance (Snedecor and Cochran 1967). Pooled data were analysed using the two way analysis of variance for unequal subclass numbers of Kempthorne (1952).

Results

Biotin levels in feed and blood plasma

The mean total biotin content of the raw materials and complete diets is summarised in Table 2. The total biotin level in the raw materials was lower than would have been estimated from published data. Moreover, the variation between samples was considerable. In the case of barley and white fishmeal the best samples contained 300 per cent more biotin than the poorest samples. The range of values recorded for complete feeds was not so great.

Plasma biotin levels in sows were influenced by dietary treatment (Table 3). The mean plasma biotin level in supplemented sows was almost twice that of the controls and no sample contained less than 65 ng biotin/100 ml. There was some evidence that lactation further reduced biotin levels in control sows but not in supplemented animals.

TABLE 4: Effect of dietary biotin supplementation and age of sow on litter performance

Parity	Treatment means (overall, parities 1 to 4)										Standard error of a difference between treatment means (se_D)				
	Overall		1		2		3		4		Overall	1	2	3	4
	C	S	C	S	C	S	C	S	C	S					
Mean number of piglets															
Born	10·8	10·9	10·0	9·3	9·5	10·6	11·8	12·0	11·7	12·0	0·4	0·7	0·6	0·9	1·0
Born alive	10·2	10·4	9·5	8·9	9·3	10·2	11·4	11·4	10·6	11·3	0·4	0·6	0·6	0·8	0·9
Live at three weeks	8·8	9·1	8·2	7·9	8·7	9·4	9·6	9·5	8·6	9·5	0·3	0·6	0·6	0·8	0·8
Live at five weeks	8·7	9·0	8·1	7·8	8·7	9·4	9·5	9·5	8·6	9·4	0·3	0·6	0·6	0·8	0·8
Mortality as percentage of live births	—	—	14·7	12·4	6·5	7·8	16·7	16·7	18·9	16·8	—	—	—	—	—
Mean litter weight at															
Birth (kg)	14·7	15·2	12·9	12·3	14·5	15·5	13·7	16·1	15·5	17·1	0·5	0·8	0·8	0·9	1·1
Three weeks (kg)	45·0	46·1	39·9	38·2	44·9	48·8	49·8	48·9	44·9	48·9	1·5	2·8	2·9	2·9	3·3
Five weeks (kg)	73·6	76·2	62·0	62·5	75·3	80·1	81·0	79·1	75·7	83·6	2·1	4·8	4·9	0·5	6·5
Mean piglet weight at															
Birth (kg)	1·42	1·44	1·34	1·33	1·50	1·49	1·40	1·44	1·44	1·51	0·02	0·06	0·07	0·06	0·08
Three weeks (kg)	5·2	5·2	4·9	4·9	5·3	5·2	5·2	5·3	5·3	5·3	0·0	0·0	0·1	0·1	0·0
Five weeks (kg)	8·6	8·6	7·8	8·2	9·0	8·7	8·6	8·5	9·0	9·2	0·2	0·4	0·4	0·5	0·5

C control
S supplemented

TABLE 5: Effect of treatment on litter productivity of sows completing four reproductive cycles (cumulative production over first four parities)

	Control	Supplemented	se$_D$
Number of animals	23	21	
Total pigs born	43·3	44·4	2·48
Total pigs weaned	34·5	37·1	1·83
Total weight of pigs born (kg)	59·1	62·5	2·60
Total weight of pigs weaned (kg)	288·2	315·3	12·66*

*Difference significant at P < 0·05

TABLE 7: Effect of biotin supplementation on sow production per day and per year†

	Control	Supplemented	se$_D$
Pigs born/sow/year	23·08	24·05	± 1·45
Pigs weaned/sow/year	18·58	20·00	± 1·02
Weight of pigs born/sow/year (kg)	31·52	34·14	± 1·70
Weight of pigs weaned/sow/year (kg)	153·64	170·97	± 7·42*

† Calculated from herd entry at 170 days of age to weaning of fourth litter for sows completing four parities only
* Difference significant at P < 0·05

Reproductive performance

Of the 80 gilts allocated to the trial 44 completed four reproductive cycles. The number of control and supplemented sows completing each of the four reproductive cycles were, respectively: 36 and 32; 30 and 29; 26 and 23; 23 and 21. Of the sows failing to complete the trial, 10 sows (eight control, two supplemented) were removed because of anoestrus or repeated failure to conceive. Sixteen sows (four control, 12 supplemented) were removed because of progressive paralysis caused by abscesses in the shoulder, hip or spine. These latter problems arose from injuries sustained during fighting after weaning and falls during mating and could not be attributed to the dietary treatment.

Neither age at onset of puberty nor at first mating were influenced by treatment. The mean interval from introduction to the boar to puberty and to effective mating was 11·0 ± 2·1 and 35·8 ± 2·6 days respectively for supplemented gilts and 11·8 ± 1·9 and 36·5 ± 2·1 days respectively for control gilts.

The effects of treatment on litter performance are summarised in Table 4. In all but the first parity, supplemented animals farrowed and reared to weaning more piglets than control sows. Over the four parities supplemented sows farrowed 0·2 more pigs per litter and weaned 0·3 more pigs per litter than the control animals. However, none of the treatment differences were statistically significant. Litter weights at birth, three weeks and weaning were respectively 0·5 kg (3·4 per cent), 1·1 kg (2·4 per cent) and 2·6 kg (3·5 per cent) greater for the supplemented animals but once again the differences failed to reach statistical significance.

When only those sows completing four litters are considered (Table 5) it is found that supplemented sows weaned 2·6 ± 1·83 more pigs than control animals and that the total weight of pigs weaned was 27·1 ± 12·7 kg greater (P < 0·05).

Supplementation of the diets with biotin had a significant effect on the post weaning performance of sows (Table 6). Pooled data for the first three weanings showed that the biotin supplemented sows returned to oestrus 2·9 ± 1·7 days sooner than the controls and conceived 6·1 ± 1·4 days sooner (P < 0·05). Not only did the supplemented sows have shorter mean intervals to conception but also a higher percentage of conceptions occurred within 10 days of weaning (81 per cent vs 72 per cent).

Considerably more control sows failed to return to oestrus within 29 days of weaning and were induced using gonadotrophin; 7·3 per cent of supplemented sows were treated in this way compared with 17 per cent of control animals. For sows returning to oestrus within 29 days of weaning, conception rate to first service was 100 per cent and 95·2 for supplemented and control animals respectively. The overall conception rate to first service, including those sows in which oestrus was induced, was 97·4 per cent for the supplemented group and 88·7 per cent for the controls.

Using the data from those sows which completed four reproductive cycles it is possible to calculate daily productivity from first mating to weaning of the fourth litter (Table 7). The weight of piglet produced per day was significantly (P < 0·05) greater in the supplemented animals. Expressing the results in terms of annual productivity shows that biotin supplementation of the diets resulted in the production of 1·42 ± 1·02 more pigs per sow per year and 17·3 ± 7·4 kg more weaned pig per sow per year (P < 0·05).

Supplementation of the diets with biotin had no significant effect on the liveweight change of the sows (Table 8).

TABLE 6: Effects of biotin supplementation on interval from weaning to oestrus and weaning to effective service

| | Treatment means (overall, parities 1 to 3) | | | | | | | | Standard error of a difference between treatment means | | | |
| | Overall | | 1 | | 2 | | 3 | | | | | |
	C	S	C	S	C	S	C	S	Overall	1	2	3
Interval from weaning to oestrus (days)	11·9	9·0	16·2	11·8	13·3	8·7	6·8	6·8	1·7	3·6	3·4	1·4
Sows returning to oestrus within 10 days of weaning (%)	74·6	83·2	63	73	68	88	96	91	—	—	—	—
Interval from weaning to conception (days)	16·5	10·4	21·9	14·9	17·6	9·9	10·0	6·9	1·4*	6·0	5·4	4·0
Sows conceiving within 10 days of weaning (%)	71·8	80·6	57	68	66	88	85	91	—	—	—	—
Sows treated for anoestrus† (%)	17·0	7·3	33·3	13·0	14·3	6·9	0·0	0·0	—	—	—	—
Sows conceiving at first service‡ (%)	95·2§	100·0§	97·0	100·0	96·5	100·0	92·6	100·0	—	—	—	—

* Means differ significantly at P < 0·05
C Control, S supplemented
† Sows not returning to oestrus within 29 days of weaning treated with PG600 (see text)
‡ Sows returning to oestrus within 29 days of weaning
§ Includes returns to service at first post pubertal oestrus

TABLE 8: Mean (± se) weight of sows (kg) throughout trial

Event	Control	Supplemented
Start of trial	26·0	
Entry to sow yard	81·8 ± 1·5	81·3 ± 1·2
Puberty	88·7 ± 1·5	89·6 ± 1·8
First service	106·4 ± 1·5	106·6 ± 1·6
First parity		
Prefarrowing	154·4 ± 1·9	155·4 ± 2·2
Postfarrowing	141·2 ± 5·0	147·4 ± 1·9
Weaning	141·4 ± 1·9	141·7 ± 1·7
Second parity		
Prefarrowing	184·2 ± 2·7	182·1 ± 2·8
Postfarrowing	173·5 ± 3·0	175·8 ± 3·2
Weaning	168·3 ± 2·5	162·2 ± 2·4
Third parity		
Prefarrowing	200·1 ± 4·3	197·1 ± 3·4
Postfarrowing	195·3 ± 7·9	187·3 ± 3·8
Weaning	174·3 ± 3·7	169·7 ± 3·5
Fourth parity		
Prefarrowing	200·4 ± 4·9	202·7 ± 3·4
Weaning	167·3 ± 12·6	180·9 ± 5·9

Discussion

The principal source of biotin for the pig is the feed which it receives. Feed materials vary greatly in their biotin content and in the biological availability of that biotin (Frigg 1976). In the trials noted in the introduction the basal diets were calculated to contain from 29 to 155 μg available biotin/kg. In recent years changes in feed formulation, feeding practices and increased sow productivity have resulted in a reduction in the quantity of biotin supplied to sows per unit of production. Brooks (1978) estimated that for sows in the United Kingdom the available biotin intake per kg weaner produced probably fell by about 27 per cent between 1967 and 1976. He further argued that this reduction in intake could have been sufficient to induce marginal deficiencies in some sows and herds.

In the current trial the basal diets contained an estimated 31 μg of available biotin/kg and assays of blood plasma indicated that some of the sows fed the basal diets had levels below the 50 ng/100 ml level which can be considered indicative of an inadequate supply (F. Tagwerker, personal communication).

There was a suggestion that the high demands of lactation, when considerable quantities of biotin are required to support fat synthesis and for excretion in the milk, further reduced plasma biotin levels of control animals. Supplementation of the diets with 350 μg biotin/kg resulted in higher plasma biotin levels and these were maintained through the lactation period.

The data obtained in this trial confirm that the reproductive performance of sows is influenced by their biotin status. It is interesting that the beneficial effects were not apparent until after the first lactation, a finding which is in agreement with the results of Penny and others (1981). However, Michel and Mastachi (1981) found a significant 14 per cent increase in the litter size of gilts when a basal diet containing an estimated 74 μg available biotin/kg was supplemented with 200 μg biotin/kg. Easter and others (1979) also reported an improvement in gilt litter size from 8·5 to 9·2 pigs born and from 7·4 to 8·5 pigs weaned when gilts fed on a corn/soya diet were supplemented with 200 μg biotin/kg; in this case the differences were not statistically significant.

An adequate supply of biotin during and immediately after the first lactation appears to be of critical importance as shown by the differences in the interval from weaning to conception and the percentage of gilts treated for anoestrus on the two dietary treatments. Indeed it would appear that from the first lactation onwards biotin status played an important part in maintaining breeding regularity.

The effects of biotin supplementation on the maintenance of breeding activity was almost certainly underestimated in this experiment. The practice of inducing oestrus with gonadotrophin in sows which had not returned to oestrus within 29 days of mating meant that fewer extended intervals were recorded for control sows than would have been the case had induction not been used.

The findings that biotin supplementation reduces the weaning to oestrus interval and improves conception rate confirm the earlier results obtained in the same unit (Brooks and others 1977).

Although they did not reach statistical significance there were consistent indications that a number of other reproductive characteristics were also improved by supplementation with biotin. For those sows which completed four parities, piglet production was increased by 1·42 pigs/sow/year. When the data for all sows in each parity was used to calculate an estimate of annual productivity the difference between treatments increased to 1·8 pigs/sow/year. Much of the difference between these estimates could be accounted for by longer weaning to remating intervals in the control sows; which also had a higher incidence of anoestrus and conception failure than the supplemented animals. When analysis was restricted to those animals completing four parities, supplementation still produced a significant increase in the weight of weaner produced/sow/year (17·3 kg, P < 0·05).

Although in this trial biotin supplementation produced biologically and economically significant improvements in sow productivity the design of the trial does not permit any conclusion on the biotin requirement of the sow. Brooks (1978) extrapolated from work on poultry and suggested that the dietary requirement of the sow was probably within the range of 140 to 540 μg biologically available biotin/kg feed. Without supplementation the majority of European diets would provide less available biotin than the lower of these figures. There is experimental evidence to suggest that 140 μg/kg feed is inadequate to support maximum reproductive performance. Robres Serrano (1981) obtained a 4·3 per cent increase in litter size when basal diets containing 103 to 155 μg available biotin/kg were supplemented with 200 μg biotin/kg. However, this improvement was not statistically significant. Michel and Mastachi (1981) added either 100 or 200 μg biotin/kg to basal diets containing approximately 74 μg available biotin/kg and found significantly more piglets born to the gilts fed the higher level of supplementation. These results suggest that the requirement level probably exceeds 175 μg available biotin/kg.

Finally it should be noted that in the recent trial of Penny and others (1981) supplementation of a basal diet, calculated to provide 56 μg available biotin/kg, produced significant improvements in litter size in second and fourth parity sows and an improvement in third parity sows which only just failed to reach statistical significance. The daily supplementation allowances in that trial were 1160 μg biotin/sow/day in pregnancy and 2320 μg biotin/sow/day in lactation.

The evidence of these recent studies indicates that diets formulated from commonly used raw materials may well fail to provide sufficient biotin to maintain maximum reproductive output in sows. Although definitive results are lacking it would appear that the requirement is in excess of 175 μg available biotin/kg diet and that higher levels may be indicated particularly during lactation and post weaning.

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The effect of different levels of dietary biotin intake on the hoof horn hardness of the gilt.

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Work previously reported from Seale-Hayne College has given some indication of the character of the biotin-deficient hoof horn. Compressive yield strength tended to be less with lower dietary biotin intake which indicated the resistance of hoof horn to traumatic injury may have been reduced. It was also reported that the firmness of hoof horn may be measured using a Durometer (Shore Manufacturing Company). The Durometer is a hand held device which was developed to assess the hardness of rubber and rubber-like material. It is operated by pressing a probe against the material to be tested. The hardness is measured as the resistance of the test material to penetration by the probe and this is recorded on a scale.

The object of the trial reported here was to assess the hardness of the toe and side-wall areas of hoof horn tissue using a Durometer and investigate whether hardness is influenced by dietary biotin intake.

Four replicates, each of eight gilts, were randomly allocated at 20kg liveweight, to one of four dietary treatments:

A. Basal diet + 5% dried egg white;

B. Basal diet;

C. Basal diet + 720 µg biotin/kg;

D. Basal diet + 720µg biotin/kg for six weeks changing to the dietary regime of treatment B for the remainder of the trial.

The basal diet was formulated from commonly-used raw materials which have been demonstrated to have a low available biotin content (Table 1).

Table 1. Percentage composition and biotin availability of basal diet

Ingredient	Diet %	Assumed biotin level (µg/kg)	Total biotin (µg/kg)	Assumed availability	Available biotin (µg/kg)
Barley	26.3	150	39	30	11.7
Wheat	48.5	100	48	10	4.8
Wheatfeed	13.2	310	40	10	4.0
Fishmeal	10.1	110	11	30	3.0
Min/Vit	2.0			TOTAL	23.4

Treatment A was a negative control diet. The egg white supplied avidin calculated to be sufficient to inactivate the biotin present in the basal diet. The gilts were individually fed once daily, on a scale related to metabolic body weight. Gilts on treatments B, C and D were fed at the rate of 93g/kgW$^{0.75}$. Gilts on treatment A were fed to a 5% higher scale to ensure that the daily nutrient intake was at least equal to that on the other treatments.

The gilts were slaughtered on reaching 80kg liveweight. The hooves were severed from the carcass above the coronet prior to scalding and deep frozen. They were subsequently defrosted and tested with the Durometer. A series of readings were taken on the outer side-wall of the hoof horn along a line bissecting the coronet and volar edge. At the leading edge two further readings were taken 5mm dorsal and ventral to the central measurement. Two measurements were taken on the median region of the toe volar surface 5mm apart.

A single gilt from treatment B died during the trial, all others completed the trial. Performance data are summarised in Table 2.

Table 2. Effect of treatment on plasma biotin level and performance

Parameter	Treatment A	B	C	D	s.e.d.	Level of significance of treatment effects	One-way analysis of variance at 5% level[1]
Daily liveweight gain (g/day)	636	568	578	562	19	**	ABCD
Feed conversion ratio	2.68	2.73	2.77	2.72	0.05	NS	
Plasma biotin level < 43 days	28	69	744	718	277	***	ABCD
(ng/100ml) > 43 days	168	221	1019	275	89	***	CABD

[1] Means in this and Table 4, joined by a line do not differ significantly (P < 0.05)

Treatment A gilts grew significantly faster than the other treatments which may be explained by the higher energy and crude protein intake provided by the scale of feeding on this treatment. Feed conversion ratio was not significantly influenced by treatment.

Results for the Durometer measurements are summarized in Tables 3 and 4. Higher values indicate increased resistance to penetration or increased "hardness". Incomplete sets of measurements were obtained from the toe region as it proved to be difficult to use the instrument effectively on the small area of hoof present in pigs of this age. The side-wall did not present such practical difficulties, but as the Durometer does not operate correctly when applied to cracks, measurements which would have been made at sites where a lesion existed were discarded.

Table 3   Comparison of inner and outer claw hardness assessed using a "Durometer"

Claw	Region	Durometer reading Inner claw	Outer claw	s.e.d.	level of significance
fore	(side-wall	73.5	72.3	0.5	*
	(toe	59.7	60.6	1.1	NS
hind	(side-wall	73.9	72.3	0.4	***
	(toe	57.4	59.4	1.2	NS

When treatments were combined, the side-wall of the inner claw had significantly higher readings than the side-wall of the outer claw for both fore and hind claws. Hardness measurements in the toe region exhibited an opposite but non-significant trend. Higher readings were obtained from the side-wall than the toe regions.

Table 4   Effect of dietary treatment on claw hardness assessed using a "Durometer"

Claw	Region	Treatment A	B	C	D	s.e.d.	level of significance of treatment effects	One-way analysis of variance (5% level)
fore inner	(side-wall	72.5	73.1	74.7	73.8	0.9	NS	
	(toe	58.5	60.9	59.8	59.8	2.8	NS	
fore outer	(side-wall	70.5	72.7	73.7	72.2	0.9	**	ADBC
	(toe	58.6	62.0	62.4	59.4	2.7	NS	
hind inner	(side-wall	73.8	74.4	74.1	73.6	0.9	NS	
	(toe	51.7	56.3	61.2	66.6	1.4	***	ABCD
hind outer	(side-wall	71.4	73.0	73.3	71.6	0.8	*	ADBC
	(toe	61.6	58.8	61.8	55.4	1.7	**	ADBE

The side-wall of claws generally exhibited lower measurements for treatment A and higher measurements for treatment C, with treatments B and D being intermediate. The results for the toe region showed a similar trend except for the hind outer claw, in which the treatment A gilts had high values. The toe measurements were more variable than the side-wall readings.

The results obtained indicate that plasma biotin level responds quickly to changes in the dietary provision of the vitamin. Withdrawal of biotin supplementation after six weeks on trial (Treatment D) resulted in an immediate reduction in plasma biotin concentration to the level found in the animals which had received no supplementation (Treatment B).

Hoof hardness as measured using the Durometer corresponded well with the plasma biotin level. Pigs which received higher intakes of dietary biotin showed an increase in hoof hardness. The withdrawal of biotin in treatment D resulted in hoof hardness values similar to those obtained in unsupplemented animals. This indicates that hoof hardness can be influenced by biotin after the horn tissue has been formed. The data also indicate that hoof hardness is affected by changes in biotin status which are not affecting the growth rate of the animal.

The Durometer provides a practical and non-destructive technique for assessing hoof horn hardness and can be used on live animals as well as on morbid material. However, the values obtained cannot at present be correlated with any measure of hoof "strength" or durability. Whilst the instrument is useful for making comparisons between populations of animals under experimental conditions the between animal variability would suggest that its value as a diagnostic tool on commercial units would be limited.