A biochemical explanation for the fatty liver and kidney syndrome of broilers: its alleviation by the short-term use of dietary fat

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1. Fatty liver and kidney syndrome (FLKS) was induced in young broiler chickens by giving them a diet composed principally of wheat and meat meal.

2. FLKS resulted in reduced growth and increased liver weight; fasting for 18 h increased mortality, liver lipid and the specific activity of hepatic ATP-citrate lyase compared with birds fed on a commercial diet. The specific activities of hepatic fructose-1,6-diphosphate-1-phosphohydrolase and pyruvate carboxylase were reduced in birds suffering from FLKS and fasted for 18 h.

3. Feeding of the FLKS-inducing diet supplemented with 150 g animal tallow/kg for 54 h considerably reduced mortality while restoring liver composition and enzyme activities towards those observed in birds fed a commercial diet. Investigations indicated that the glycerol component of the fat was not responsible for the observed responses.

4. The present results suggest that in FLKS insufficiencies of biotin are induced in specific enzyme systems, but the syndrome may be alleviated without the use of supplementary biotin.

5. The evidence indicates that, when stressed, birds affected by FLKS die from the hypoglycaemia occurring as a result of a reduced capacity for gluconeogenesis.

Fatty liver and kidney syndrome (FLKS) can cause considerable mortality in growing broilers and has been reported to be particularly prevalent when birds are given diets composed principally of wheat and meat meal (Payne, Gilchrist, Pearson & Hemsley, 1974) although the syndrome also occurs with other diets (Blair & Whitehead, 1974). Reports indicate that the syndrome results from low dietary biotin concentrations and low body reserves of biotin in combination with stress (Whitehead, Blair, Bannister & Evans, 1975; Johnson, Hood, Pearson & Fogerty, 1976), so that a biotin insufficiency is implicated. This implication is strengthened by the fact that hepatic pyruvate carboxylase activity is extremely low in FLKS-affected birds and it has been suggested that birds die from the resulting hypoglycaemia induced by stress factors such as fasting (Johnson *et al.* 1976; Whitehead, Bannister, Evans, Siller & Wight, 1976). However, the pattern of some enzyme activities in the livers of FLKS-affected birds differs from that in birds made biotin-deficient by the feeding of purified diets without biotin. In particular, biotin deficiency reduces the specific activity of acetyl CoA carboxylase (Mason & Donaldson, 1972) and hepatic ATP-citrate lyase (Balnave, 1975*a*), whereas in FLKS-affected birds the activity of acetyl CoA carboxylase and hepatic lipogenesis are increased (Johnson *et al.* 1976).

In the studies now reported an attempt was made to clarify the importance of biotin in the development of FLKS. Since lipogenesis and the activity of acetyl CoA carboxylase are substantially increased in the livers of affected birds it was hypothesized that under certain conditions this enzyme, which is biotin-dependent, is stabilized thereby utilizing biotin and decreasing the availability of biotin for other dependent enzymes such as pyruvate carboxylase. Thus procedures that release biotin from acetyl CoA carboxylase might alleviate FLKS. The results presented indicate that this was accomplished by the feeding of high levels of dietary fat.

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EXPERIMENTAL

Four-week-old broiler pullets (Allied Genetic Breeders) which had been obtained at 1-d-old from young broiler breeder stock and kept in a brooder without heat from 2 weeks of age were used in the present studies. Apart from the second experiment, where the complete diets were fed from 1-d-old, birds were fed crushed wheat alone for the first 4 d of life and then either a commercial chick starter diet or a diet similar to that described by Payne *et al.* (1974) and composed of (g/kg) wheat 790, meat and bone meal 200, D,L-methionine 1·4 and L-lysine 2·5, with normal levels of trace nutrients, excluding biotin.

Expt 1

Initially in this study part of the meat meal was heated at 100° for 30 h before being incorporated into separate diets. However, the mortality resulting from an 18 h fast at day 28 for birds fed on this heated meat meal was less than that recorded for birds fed the unheated meat meal (13 % *v.* 31 %; 45 birds/treatment) and so further experimental work was confined to birds given diets containing the original, unheated meat meal.

At 28 d of age forty-five randomly selected pullets from larger populations given each diet were fasted overnight for 18 h at room temperature (20°) and mortalities were recorded the following morning. Four fed birds randomly selected from each treatment were also killed at this time. Blood was collected in heparinized tubes, the plasma separated by centrifugation and plasma glucose determined on an autoanalyser by the glucose oxidase technique of Huggett & Nixon (1957). Determinations were also made of liver lipid content (Folch, Lees & Sloane Stanley, 1957) and the specific activity of hepatic ATP-citrate lyase (*EC* 4.1.3.8). The method of enzyme assay was essentially the same as that described by Balnave & Pearce (1976).

At 32 d of age half the remaining birds given the wheat and meat meal diet were transferred to a separate brooder compartment and given the same diet to each kg of which had been added 150 g high-grade animal tallow. Four fed birds were randomly selected and killed 24 h later from this treatment group and from birds maintained on the commercial diet, and plasma glucose, hepatic lipid and ATP-citrate lyase determinations made. Groups of birds from all three dietary treatments were also fasted overnight for 18 h at room temperature 30 and 54 h respectively after the introduction of the fat-supplemented diet. Mortalities were recorded at the end of the fasting period and some of the surviving fasted 54 h fat-fed birds taken for the determination of plasma glucose, liver lipid and liver fatty acid composition and hepatic ATP-citrate lyase, fructose-1,6-diphosphate 1-phosphohydrolase (*EC* 3.1.3.11) and pyruvate carboxylase (*EC* 6.4.1.1) (see Balnave & Pearce, 1976). Similar determinations were made in fasted birds previously given the commercial and wheat and meat meal diets.

In addition, twenty-one birds that had been previously given the wheat and meat meal diet and fasted for 18 h were randomly distributed into four groups and given the following: (i) 8 IU ox-insulin (Sigma Chemical Co.) in 0·5 ml 0·15 M-NaCl (Langslow, Butler, Hales & Pearson, 1970) (six birds); (ii) 50 μg glucagon (Sigma Chemical Co.) in 0·5 ml 0·15 M-NaCl (Langslow *et al.* 1970) (six birds); (iii) 0·5 ml 0·15 M-NaCl (three birds); (iv) 400 mg glucose in 1 ml distilled water (six birds). In addition treatments (i) and (iii) were each given to three fasted birds previously fed on the commercial diet. These treatments were given as two injections 2 h apart; the first injection was given into the jugular vein and the second into the breast muscle. After a further 1 h mortalities were recorded and blood samples were taken from the survivors which were then killed for estimations of liver enzymes and liver lipid.

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Expt 2

The same batch of dietary ingredients and the same procedures were used as in the first experiment except that the diets were introduced at 1 d of age and the mean environmental room temperature was 30°. No mortalities were observed after an 18 h fast at day 28 and physical symptoms of biotin deficiency were not as severe as in the first experiment. This experiment was therefore abandoned.

Expts 3 and 4

New batches of dietary ingredients were obtained and used for these two experiments. The birds were initially fed crushed wheat for 4 d before the commercial and wheat and meat meal diets were introduced. From 2 weeks of age birds were kept at 20° in a temperature controlled room.

Expt 3 was carried out in a similar fashion to Expt 1. Randomly selected birds given each of the above diets were fasted overnight for 18 h on day 22. The wheat and meat meal diet supplemented with 150 g tallow/kg was then given. Overnight fasting for 18 h was carried out on day 26 when randomly-selected groups of birds previously given the wheat and meat meal diet had received the fat-supplemented diet for either 30 or 54 h. Since a number of the birds affected by FLKS became lethargic when fasted and subsequently refused to eat when offered food, all groups were given the commercial starter diet containing adequate biotin at the end of the fasting period. Total mortalities in Expts 3 and 4 were recorded during the 18 h of fast and the first 12 h of re-feeding. At the end of the fasting period in Expt 3 surviving birds from each treatment that were judged to be either affected or unaffected by FLKS were killed for the determination of the specific activities of hepatic ATP-citrate lyase and fructose-1,6-diphosphate 1-phosphohydrolase.

In Expt 4 250 birds were maintained on the wheat and meat meal diet for 30 d. They were then randomly distributed into seven groups and continued to be fed on either the wheat and meat meal diet (two groups) or this diet supplemented with 15 % (w/w) high grade animal tallow (two groups) or 2 % (w/w) glycerol (one group) or biotin (0·30 mg/kg) (two groups). Three groups receiving respectively the wheat and meat meal diet and this diet supplemented with either biotin or fat were used in a 6 d feeling trial before fasting overnight for 18 h. The remaining four groups were used to examine the effects of such a fast on the mortality of birds fed the wheat and meat meal diet supplemented with fat, glycerol or biotin for 54 h. A group of twenty-five birds fed throughout on a commercial diet and fasted overnight for 18 h at the same time as the groups on the 6 d feeding trial was used for comparative purposes.

The results were compared using analysis of variance and Student's *t* test. Log transformations were taken where there was evidence of variance heterogeneity as indicated by a comparison of the treatment means with the standard error of the raw data. Grouped mortality results were analysed by the Chi-squared test.

RESULTS

The determined chemical composition of the meat meals and experimental diets are given in Table 1. A commercial chick starter diet was used instead of a broiler starter diet so as to obtain a crude protein (N × 6·25) level similar to that expected from the wheat and meat meal diets. The commercial diet differed markedly from the wheat and meat meal diet used in Expt 1 only in having more biotin, leucine, isoleucine and threonine and less lysine. In Expts 3 and 4 a poorer quality meat meal was used and although the crude protein

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Table 1. *Determined chemical composition of meat meals and experimental diets (g/kg diet) fed to broiler pullets*

	Commercial diet	Meat meal		Wheat and meat meal diet*	
		Expt 1	Expts 3 and 4	Expt 1	Expts 3 and 4
Determined biotin ($\times 10^{-6}$)†	222	—	—	144	110
Determined crude protein (N × 6·25)	172	505	390	202	172
Determined ether extract	37	120	95	35	30
Alanine	10·7	39·2	41·6	11·3	10·6
Arginine	9·9	35·0	33·1	10·3	9·6
Aspartic acid	13·1	36·9	27·2	11·5	8·1
Glutamic acid	38·9	60·4	50·0	39·2	35·9
Glycine	13·3	68·3	61·0	16·9	15·0
Histidine	4·9	9·1	8·4	4·3	3·0
Isoleucine	7·4	13·0	10·3	5·4	5·0
Leucine	14·1	33·9	21·8	12·8	10·3
Lysine	8·2	24·9	22·2	10·3	7·0
Methionine	4·5	7·8	6·3	4·2	3·3
Phenylalanine	9·1	21·1	11·8	8·4	7·0
Proline	15·7	47·2	50·7	19·1	18·7
Serine	7·1	15·9	14·5	6·4	6·2
Threonine	7·0	14·8	13·4	5·1	4·8
Tyrosine	5·1	12·5	7·0	5·5	4·9
Valine	6·7	14·4	10·6	6·4	5·0

* Contained the following added micronutrients (/kg): retinol, 1·8 mg; cholecalciferol, 62·5 μg; DL-α-tocopherol, 8 mg; menaphthone, 2 mg; nicotinic acid, 25 mg; pantothenic acid, 10 mg; folic acid, 1·5 mg; riboflavin, 5 mg; vitamin B_{12}, 7 μg; Mn, 80 mg.

† Hood (1975).

concentration of the resulting wheat and meat meal diet was similar to that of the commercial diet the protein quality was much poorer.

Birds fed on the commercial diet and fasted overnight on day 28 (Expt 1) and day 22 (Expt 3) were respectively, 24 % and 38 % heavier than similarly fasted birds fed on the wheat and meat meal diet. None of the birds fed on the commercial diet died as a result of the fast, but in Expt 1 fourteen from forty-five and in Expt 3 twelve from forty-five fed on the wheat and meat meal diet died. Fasting greatly increased the mortality of birds given the wheat and meat meal diet. The mortality during the 18 h fasting period was double the total mortality which occurred during the previous 2 weeks when birds had free access to food. Birds fed on the wheat and meat meal diet had severe biotin deficiency symptoms similar to those observed by Whitehead *et al.* (1976) and by the senior author in previous studies using purified, biotin-free diets. Severe haemorrhagic fissuring of the feet and dermatitis around the beak were consistent symptoms. Perosis was observed in some FLKS-affected birds.

As shown in Table 2, fully-fed birds given the wheat and meat meal diet in Expt 1 had significantly larger livers and a significantly greater hepatic ATP-citrate lyase activity than birds fed on the commercial diet. Feeding animal tallow for 24 h substantially reduced liver weight in fully-fed birds given the wheat and meat meal diet, although liver weight was still significantly greater than in birds given the commercial diet. ATP-citrate lyase activity was reduced by fat feeding nearly to that observed in birds given the commercial diet.

The results in Table 3 indicate that the high mortality observed after an 18 h fast in birds given the wheat and meat meal diet in Expts 1 and 3 was reduced by 54 h but not 30 h of fat feeding. A Chi-squared test indicated that the mortality was signi-

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Table 2. *Mean plasma glucose concentrations, liver weight, liver lipid and hepatic ATP-citrate lyase activities in fully-fed birds in Expt 1*†

(Four birds/treatment)

Age (d)		Plasma-glucose (mg/100 ml)	Liver weight (g/100 g body-wt)	Liver lipid (mg/g liver)	ATP-citrate lyase‡
28	Commercial diet	279	2·77	33·5	15·5
	Wheat and meat meal diet	314**	4·35*	34·1	51·4*
	SEM (6 df)	5·7	0·342	2·68	7·09
33	Commercial diet	288	2·69	38·0	15·4
	Fat-supplemented wheat and meat meal diet fed for 24 h	287	3·75*	42·7	17·2
	SEM (6 df)	4·7	0·243	2·78	2·03

† For details see p. 320.
‡ Specific activity expressed as nmol substrate metabolized/min per mg protein in the extract.
Values significantly different to those of birds fed the commercial diet: * $P < 0·05$, ** $P < 0·01$.

Table 3. *The number of deaths resulting from an* 18 *h fast in Expts* 1 *and* 3*

Expt	Age (d)	Commercial diet	Wheat and meat meal diet	Fat-supplemented wheat and meat meal diet fed for: 30 h	54 h
1	33	0/20†	8/20	8/20	—
	34	0/16	7/26	—	0/16
3	26	2/30	11/30	12/30	3/30

* For details, see pp. 320–321.
† No. of deaths/no. of birds fasted.

ficantly influenced by treatment in Expt 1 (day 33, $P < 0·05$; day 34, $P < 0·001$) and Expt 3 ($P < 0·01$).

Birds given the wheat and meat meal diet in Expt 1 had significantly ($P < 0·001$) larger livers and liver lipid levels than birds given the commercial diet; the specific activity of ATP-citrate lyase was significantly ($P < 0·01$) increased and fructose-1,6-diphosphate 1-phosphohydrolase ($P < 0·001$) and pyruvate carboxylase ($P < 0·01$) activities significantly reduced in the livers of these birds (Table 4). Supplementation of the wheat and meat meal diet with tallow for 54 h resulted in significant ($P < 0·001$) reductions in liver weight and lipid content and in the specific activity of ATP-citrate lyase ($P < 0·05$) in birds fasted for 18 h. No significant differences were observed between birds given the fat-supplemented diet and birds given the commercial diet apart from a slight reduction ($P < 0·05$) in the specific activity of fructose-1,6-diphosphate 1-phosphohydrolase in birds given the fat-supplemented diet. The reduction in the specific activity of ATP-citrate lyase as a result of fat-supplementation of the wheat and meat meal diet corresponded with significant increases in the specific activities of fructose-1,6-diphosphate 1-phosphohydrolase ($P < 0·001$) and pyruvate carboxylase ($P < 0·05$).

The results shown in Table 5 indicate that, in comparison with the commercial diet, giving the wheat and meat meal diet significantly altered the liver concentrations of all fatty acids other than palmitic acid (C16:0). Supplementing this diet with animal tallow for 54 h significantly altered the liver fatty acid concentrations towards those observed in birds given the commercial diet.

In the present study the administration of insulin to six fasted birds previously given the FLKS-inducing diet resulted in four deaths within 3 h. Most of the deaths occurred within

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Table 4. *The effect of* 54 *h fat-feeding on the mean plasma glucose concentrations and liver responses of birds subjected to an* 18 *h overnight fast at* 33 *d of age in Expt* 1

(Four birds/treatment)

	Plasma glucose (mg/100 ml)	Liver weight (g/100 g body-wt)	Liver lipid (mg/g liver)	ATP-citrate lyase†		Fructose-1,6-diphosphate 1-phosphohydrolase†		Pyruvate Carboxylase‡	
				Original data	Transformed data	Original data	Transformed data	Original data	Transformed data ($\times 10^4$)
Commercial diet	225	2·50	38·5	6·0	0·741	51·6	1·702	0·135	3·076
Wheat and meat meal diet	154	4·56***	94·6***	27·0	1·418**	10·4	0·999***	0·020	1·437**
Fat-supplemented wheat and meat meal diet fed for 54 h	228	2·69	44·7	11·0	0·976	29·5	1·456*	0·068	2·709
SEM (9 df)	31·9	0·182	5·39		0·106		0·062		0·281

† Specific activity expressed as nmol substrate metabolized/min per mg protein in the extract.
‡ Specific activity expressed as μmol bicarbonate fixed/min per mg protein in the extract.
Values significantly different from those of birds fed the commercial diet: *$P < 0·05$; **$P < 0·01$; ***$P < 0·001$.

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Table 5. *Liver fatty acid composition (mg/g fatty acids) of fasted birds in Expt* 1

(No. of birds/treatment given in parentheses)

	Commercial diet (4)	Wheat and meat meal diet (3)	Fat-supplemented wheat and meat meal diet fed for 54 h (4)	SEM (8 df)
C14:0	83	25***	72	6·6
C16:0	223	235	217	11·8
C16:1	10	166***	29	9·6
C18:0	245	55***	240	21·2
C18:1	120	421***	178	27·2
C18:2	138	71***	105*	9·3
C20:4	91	13**	94	12·6
C22:6	65	0	20*	9·35†

Values significantly different to those of birds fed the commercial diet: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† Calculated from the data for birds fed the commercial and fat-supplemented wheat and meat meal diets (6 df).

Table 6. *The mortality resulting from an* 18 *h fast and the mean body-weight and feed efficiency results from Expt* 4

	Commercial diet	Wheat and meat meal diet	Wheat and meat meal diet + fat	Wheat and meat meal diet + glycerol	Wheat and meat meal diet + biotin
(*a*) Supplemented diets fed for 54 h					
Mortality after 18 h fast†	—	9/50	3/50	14/50	0/25
(*b*) Supplemented diets fed for 6 d					
Mortality after 18 h fast†	0/25	5/25	0/25	—	0/25
Daily body-wt gain (g)	—	17·8	21·8	—	21·3
Feed efficiency (g body-wt/g feed)	—	0·38	0·43	—	0·42

† No. of deaths/No. of birds fasted.

10 min of the initial injection. The administration of glucagon resulted in the death of one bird and the remaining five birds on this treatment showed no signs of recovery. No deaths occurred in birds given either the commercial diet or the FLKS-inducing diet when saline or glucose were administered. These mortality results were mirrored by the plasma glucose concentrations which indicated that insulin had a more marked effect in birds suffering from FLKS than in birds given the commercial diet. Insulin administered to the latter birds reduced the mean plasma glucose concentration to 148 mg/100 ml, whereas when insulin was given to FLKS-affected birds the mean plasma glucose concentration was reduced to 34 mg/100 ml. Glucagon administration to FLKS-affected birds resulted in a plasma glucose concentration of 164 mg/100 ml, while glucose administration increased the mean plasma glucose concentration to 406 mg/100 ml. No significant differences in hepatic enzyme specific activities were observed as a result of these treatments.

Examination of hepatic ATP-citrate lyase and fructose-1,6-diphosphate 1-phosphohydrolase in birds surviving the 18 h fast in Expt 3 indicated that, irrespective of whether fat was fed for 0, 30 or 54 h, birds from any treatment that had succumbed to the syndrome had a similar hepatic enzyme pattern to birds affected by FLKS in Expt 1. Birds, from any treatment, unaffected by the syndrome had an enzyme pattern similar to birds given the commercial diet.

The results from Expt 4 (Table 6) indicate that feeding the wheat and meat meal diet

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supplemented with glycerol for 54 h produced a high mortality whereas similar supplementation with animal tallow reduced the mortality compared with birds fed the unsupplemented wheat and meat meal diet. No deaths were observed after similar supplementation with biotin. A Chi-squared test indicated that the mortality was significantly ($P < 0.01$) influenced by treatment. Feeding the fat-supplemented diet for 6 d also eliminated mortality and improved body-weight gain and feed efficiency compared with birds given the biotin-supplemented diet. The mortality was again significantly ($P < 0.01$) influenced by treatment.

DISCUSSION

A commercial chick starter diet rather than broiler starter diet was used for comparative purposes in the present investigations since mortality from FLKS has been reported to be particularly high when diets containing low levels of protein and fat have been fed to broiler chicks (Blair & Whitehead, 1974; Blair, Whitehead & Teague, 1975). The results of Expt 4 indicate that the mortality rate on the commercial diet was similar to that on the wheat and meat meal diet which had been supplemented with biotin. In the present work FLKS was induced whether a good quality meat meal (Expt 1) or poor quality meat meal (Expts 3 and 4) was used. However, although the same dietary ingredients were used in both the first and second experiments, the incidence of FLKS was low in the second experiment and no deaths were observed after an 18 h fast at day 28. Physical symptoms of biotin deficiency were much less acute than in the other experiments. The only procedural differences between this experiment and the others, apart from the use of a different hatch of chicks, were that in this experiment birds were given the complete diets at 1-d-old and the mean environmental temperature during most of the growth period was 30° i.e. approximately 10° higher than in the other experiments. The birds in the second experiment were therefore under constant heat stress and were often observed to be panting throughout the daylight hours. However, Blair & Whitehead (1974) have reported that elevation of environmental temperature by only 3° above 'normal recommended temperature' increased mortality from FLKS by 20%.

Many of the birds affected by FLKS became lethargic during the fasting period and subsequently refused to eat when food was offered. Therefore, in Expts 3 and 4 at the end of the 18 h fast all birds were offered the commercial diet containing an adequate level of biotin and mortalities were recorded during the 18 h fast and the first 12 h of refeeding. These observations confirm an earlier report (Balnave & Pearce, 1976) that FLKS-affected birds stop eating before death and indicate that the onset of fasting may be one of the terminal symptoms of FLKS.

Feeding animal tallow for 54 h substantially reduced the mortality from FLKS. Supplementation of the diet with glycerol had no beneficial effect on mortality and indicated that this component of the fat was not responsible for the observed responses.

The hepatic enzyme studies give some indication of the metabolic factors that alleviate FLKS in birds given the fat-supplemented wheat and meat meal diet for 54 h. Hepatic lipogenesis was reduced in the fat-supplemented birds and the specific activities of the gluconeogenic enzymes were increased to approximately 50–60 % of those of birds maintained on the commercial diet. These results confirm an earlier report (Balnave & Pearce, 1976) that not only pyruvate carboxylase but also fructose-1,6-diphosphate-1-phosphohydrolase is reduced in activity in FLKS-affected birds. The increase in the specific activities of these gluconeogenic enzymes as a result of fat-supplementation of the FLKS-inducing diet was reflected in increased plasma glucose concentrations in fasted birds to levels observed in fasted birds which had been fed throughout on the commercial diet. Plasma glucose concentrations in fed birds were similar. The high mortality obtained when the

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diet was supplemented with glycerol would indicate that the increased activities of pyruvate carboxylase and fructose-1,6-diphosphate-1-phosphohydrolase were not the result of induction by the feeding of this precursor of glucose. Also 54 h feeding with these dietary glycerol levels would have had little effect on the size of the glucose pool in FLKS-affected birds.

Birds affected by FLKS normally have high liver and blood lipid levels when fasted (Evans, Bannister & Whitehead, 1975; Johnson *et al.* 1976) and the unsupplemented wheat and meat meal diets used in the present work contained 3–3·5 % fat. Nevertheless, the response to supplementary fat was rapid and the results support the original hypothesis that in this condition the biotin is preferentially retained by acetyl CoA carboxylase thereby inducing an insufficiency of biotin in other enzyme systems including, in particular, pyruvate carboxylase. This results in physical symptoms of biotin deficiency and, in certain cases, death. This effect would presumably be most noticeable where the supply of biotin was marginal and Johnson *et al.* (1976) have reported greatly reduced liver biotin levels in birds suffering from FLKS. However, Balnave & Pearce (1976) have shown that the provision of a diet low in biotin (57 μg/kg diet) does not always result in low liver biotin levels or a high incidence of FLKS in broilers, so that the incidence of FLKS would not appear to be related solely to the biotin content of the feed. Other factors such as the biotin reserve of the chick at hatching have been suggested (Payne *et al.* 1974).

The present results also explain the earlier observations of Whitehead *et al.* (1975) that long-term feeding of a FLKS-inducing diet supplemented with any one of a number of fats had a beneficial effect on mortality compared with the unsupplemented diet. The hypothesis proposed in the present report would confirm their suggestion that the effect was attributable to fat per se rather than to any particular constituent of the fat. Other reports have also indicated the benefits of long-term fat feeding (see Blair *et al.* 1975). The variation in mortality in birds fed the fat-supplemented diet for different periods of time is not unexpected since Majerus & Kilburn (1969) have reported that in rats the half-life of acetyl CoA carboxylase is 48 h.

In Expt 1, the reduction in mortality from FLKS in birds fed the animal tallow for 54 h was associated with an alteration in liver fatty acid profiles as well as hepatic enzyme activities. This was very marked in the case of palmitoleic acid which is substantially increased in both FLKS (Whitehead, 1975; Johnson *et al.* 1976) and biotin-deficiency (Balnave & Brown, 1967). Earlier studies of extended feeding with purified, biotin-free diets have shown that supplementation of such diets with 46 g arachis oil/kg reduced liver palmitoleic acid levels from 19 to 9 %, and not to the lower levels observed with dietary biotin supplementation (Balnave & Brown, 1967).

The results of the 6 d feeding trial carried out in Expt 4 suggest that as well as continuing to have a beneficial effect on reducing mortality the extended feeding of animal tallow improved body-weight gain and food conversion efficiency. In the report of Whitehead *et al.* (1976) FLKS mortality was eliminated by supplementing a low-protein diet with 150 g maize oil/kg but body-weight gain was poorer than on the unsupplemented diet. The differences in results in regard to body-weight might be related to the small numbers of birds and the limited duration of the present feeding trial or to differences in the severity of the syndrome, but other reports concerning the benefits of long-term fat feeding (see p. 327) and the experience of Australian feed manufacturers would suggest that dietary supplementation with small amounts of fat has a permanently beneficial effect on reducing the occurrence of FLKS.

FLKS is not always easy to reproduce experimentally (Balnave, 1975*b*; Pearce, 1975; Balnave & Pearce, 1976). This fact and others discussed previously would support previous suggestions by these workers that there is not a simple relationship between FLKS and

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dietary biotin content. Rather it would appear that some environmental or dietary factor is interfering with the normal utilization of biotin thereby inducing insufficiencies in specific enzyme systems. In the presence of these factors supplementary biotin presumably prevents the occurrence of these specific insufficiencies of biotin; in their absence, or when lipogenesis is naturally low because of dietary fat supplementation, the requirement for this vitamin is possibly satisfied by dietary levels much lower than suggested by recent studies (Payne *et al.* 1974; Whitehead, Bannister, Wight & Weiser, 1974). Thus in the present study FLKS was alleviated without the use of supplementary biotin. The fact that the response to fat was so rapid (54 h) would suggest that this response was to fat per se and was not a reflection of increased biotin synthesis by intestinal microflora.

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REFERENCES

Balnave, D. (1975*a*). *Int. J. Biochem.* **6**, 17.
Balnave, D. (1975*b*). *Br. J. Nutr.* **34**, 83.
Balnave, D. & Brown, W. O. (1967). *Comp. Biochem. Physiol.* **23**, 917.
Balnave, D. & Pearce, J. (1976). *Br. Poult. Sci.* **17**, 627.
Blair R. & Whitehead, C. C. (1974). *Proc. 15th Wld's Poult. Congr., New Orleans*, p. 380.
Blair, R., Whitehead, C. C. & Teague, P. W. (1975). *Res. vet. Sci.* **18**, 76.
Evans, A. J., Bannister, D. W. & Whitehead, C. C. (1975). *Res. vet. Sci.* **18**, 26.
Folch, J., Lees, N. & Sloane Stanley, C. H. (1957). *J. biol. Chem.* **226**, 497.
Hood, R. L. (1975). *J. Sci. Fd. Agric.* **26**, 1847.
Huggett, A. St. G. & Nixon, D. A. (1957). *Biochem. J.* **66**, 12*P*.
Johnson, A. R., Hood, R. L., Pearson, J. A. & Fogerty A. C. (1976). *Proc. Nutr. Soc.* **35**, 129A.
Langslow, D. R., Butler, E. J., Hales, C. N. & Pearson, A. W. (1970). *J. Endocrinol.* **46**, 243.
Majerus, P. W. & Kilburn, E. (1969). *J. biol. Chem.* **244**, 6254.
Mason, J. V. & Donaldson, W. E. (1972). *J. Nutr.* **102**, 667.
Payne, C. G., Gilchrist, P., Pearson, J. A. & Hemsley, L. A. (1974). *Br. Poult. Sci.* **15**, 489.
Pearce, J. (1975). *Br. Poult. Sci.* **16**, 565.
Whitehead, C. C. (1975). *Res. vet. Sci.* **18**, 32.
Whitehead, C. C., Bannister, D. W., Evans, A. J., Siller, W. G. & Wight, P. A. L. (1976). *Br. J. Nutr.* **35**, 115.
Whitehead, C. C., Bannister, D. W., Wight, P. A. L. & Weiser, H. (1974). *Proc. 15th Wld's Poult. Congr., New Orleans*, p. 70.
Whitehead, C. C., Blair, R., Bannister, D. W. & Evans, A. J. (1975). *Res. vet. Sci.* **18**, 100.

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