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The influence of essential fatty acids and food restriction on the specific activities of hepatic lipogenic and glutamate-metabolizing enzymes in the laying hen

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1. Extended feeding of an essential fatty acid (EFA)-deficient diet to laying hens increased liver size and liver lipid concentration.

2. The specific activities of hepatic lipogenic and glutamate-metabolizing enzymes were increased by feeding the EFA-deficient diet.

3. Restriction of daily food intake to 75 % of *ad lib.* intake did not affect the response to dietary fat concentration.

4. Hepatic enzyme activities in hens fed on restricted amounts of food and killed just before, or after, the normal daily feeding time indicated no reduced capacity for lipogenesis at the earlier time.

It has been established that avian hepatic lipid synthesis is dependent on dietary composition and that hepatic lipogenic enzyme activity is increased when low-fat diets are fed to immature and laying hens (Balnave & Pearce, 1969; Mason & Donaldson, 1972). Feeding with low-fat diets containing less than 10 g linoleic acid/kg induces an essential fatty acid (EFA) deficiency in the domestic fowl. This results in decreased reproductive performance as well as increased liver size and liver lipid content (Balnave, 1970*a*). The EFA deficiency symptoms can be overcome by feeding vegetable oils containing high levels of linoleic acid and this procedure is used to obtain maximum egg weight from laying hens.

The exact mechanism by which dietary linoleate increases egg weight is unknown. However, the response has been shown to vary, depending on whether birds are fed on a restricted intake basis or given free access to food (Balnave & Brown, 1968; Balnave, 1970*b*). Calvert (1967) and Balnave (1969) have found that the decreased egg weight from EFA-deficient hens is reflected in a decreased yolk weight and yolk lipid content. In addition, the results obtained by Balnave (1968) suggest that EFA deficiency may induce changes in egg-yolk protein fractions and Jensen (1968) has likewise suggested that EFA effects on egg weight may be related to the synthesis or transport of liver lipoproteins.

The present initial experiment was done to study hepatic lipogenic enzyme activity in laying hens maintained for an extended period on isoenergetic, semi-purified diets containing either low or high levels of linoleic acid. During this time birds were either given free access to food or their intake was restricted. As glutamic acid is important in amino acid biosynthesis, studies were also made of certain hepatic glutamate-metabolizing enzymes.

Table 1. *Composition (g/kg) of diets given to laying hens*

Ingredient	Expt 1		Expt 2
	Low-linoleate*	High-linoleate*	Conventional
Ground maize	—	—	715
Maize starch	570	410	—
Soya-bean meal	285	285	110
Fish meal	54	54	75·5
Maize oil	—	60	—
Oatfeed	—	100	—
Dried-grass meal	—	—	20
Limestone flour	65	65	65
Dicalcium phosphate	20	20	10
NaCl	3	3	2
Vitamins and minerals†	3	3	2·5
Calculated ME (Mcal/kg)	2·94	2·94	2·94
(MJ/kg)	12·30	12·30	12·30

ME, metabolizable energy.

* Semi-purified diets also contained carophyll orange (22 mg/kg) (Roche Products Ltd, Welwyn Garden City, Herts.).

† Micromix BETA 14TE (Cooper Nutrition Products Ltd, Hainault, Ilford, Essex).

The second experiment was done to obtain comparable information on the effect of the different feeding regimens on laying hens fed on conventional diets. Therefore the enzymes were also studied in the livers of laying hens maintained for an extended period on a conventional diet (isoenergetic with the semi-purified diets), fed either *ad lib.* or at a restricted intake. As restricted feeding of laying hens is used as a means of reducing body fat deposition, and since no comparative values for daily variations in the activities of lipogenic enzymes are available for birds given free access to food or fed on a restricted intake basis, the opportunity was taken to kill birds just before, and after, the normal daily feeding time of the restricted regimen.

EXPERIMENTAL

In the first experiment, 70-week-old white, hybrid laying pullets which had been maintained for 30 weeks on either a semi-purified, low-linoleate or an isoenergetic, semi-purified, high-linoleate diet were used (Table 1). Ten birds were given each of these diets, five on an *ad lib.* basis and five on a daily restricted intake of 100 g, which corresponded to a food restriction of approximately 25%. The birds receiving the restricted amount of food were fed at 09.00 hours each day while birds given free access to food were fed twice weekly at the same time of day. Birds were killed at 11.00 hours and the livers excised rapidly. Portions of liver were immediately chilled and homogenized with 4 vol. ice-cold 0·1 M-potassium phosphate buffer, pH 7·0, containing 7·0 mM-2-mercaptoethanol. The homogenates were centrifuged at 20000 *g* for 40 min at 0–5° and the resulting supernatant fractions were assayed for enzyme activities as described by Balnave (1972) and Balnave & Jackson (1974). The specific activities of the following enzymes were estimated: ATP citrate lyase (*EC* 4.1.3.8), NADP–malate dehydrogenase (L-malate: NADP oxidoreductase, *EC* 1.1.1.40), NADP–

Table 2. *Expts* 1 *and* 2. *Mean body-weights, liver weights and liver lipid concentrations for laying hens given isoenergetic low-linoleate, high-linoleate or conventional diets either* ad lib. *or at a restricted intake* (75–85% ad lib. *intake*)

Expt	Diet*	Feeding regimen	Body-wt (g)	Liver wt (g/kg body-wt)	Liver lipid (mg/g liver)
1	Low-linoleate	Restricted	1395	34·8	92·4
	High-linoleate	Restricted	1565	27·7	61·2
	Low-linoleate	*Ad lib.*	1530	36·3	113·9
	High-linoleate	*Ad lib.*	1750	29·8	76·4
	SEM (16 df)		—	2·1	13·7
2					
Pre-feeding†	Conventional	Restricted	1600	33·5	79·2
Post-feeding†	Conventional	Restricted	1520	31·0	79·2
Pre-feeding†	Conventional	*Ad lib.*	1575	31·7	77·3
Post-feeding†	Conventional	*Ad lib.*	1570	27·7	86·0
	SEM (16 df)		—	2·5	12·7

* For details of diets, see Table 1.

† Hens were killed post- and pre-feeding, 2·5 and 22·5 h respectively after a single daily feed at 11.30 hours.

isocitrate dehydrogenase (*threo*-D_s-isocitrate: NADP oxidoreductase (decarboxylating); *EC* 1.1.1.42) (ICD), alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase; *EC* 2.6.1.2), aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase; *EC* 2.6.1.1) and glutamate dehydrogenase (*EC* 1.4.1.2) (GDH). Liver lipid was determined by the method of Folch, Lees & Sloane Stanley (1957) and the results analysed by analysis of variance.

In the second experiment twenty 68-week-old laying pullets of the same strain as that used in the first experiment were used. They had been maintained for 48 weeks on a conventional layer diet (Table 1), ten on an *ad lib.* basis and ten restricted to a daily food intake of 100 g from the age at which they achieved this level of food consumption. At the time of the experiment the food consumption of the birds receiving the restricted amount of food corresponded to approximately 85% of the *ad lib.* intake. For 4 weeks before they were killed birds were fed at 11.30 hours in a similar way to the first experiment. Birds were killed at 10.00 and 14.00 hours and the subsequent procedures followed were those described for the first experiment.

RESULTS

The mean body-weights, liver weights and liver lipid concentrations for birds in both experiments are given in Table 2. Analysis of variance indicated that supplementary dietary linoleate had a significant ($P < 0.01$) influence on liver weight as a percentage of body-weight in the first experiment; liver weights were significantly ($P < 0.05$) reduced by supplementary linoleate under both feeding regimens. Restricted feeding had no significant effect in either experiment, and the time of killing was also without effect in the second experiment.

Supplementary dietary linoleate significantly ($P < 0.05$) reduced the liver lipid concentrations for the birds in the first experiment. Restricted feeding had no

Table 3. *Expts* 1 *and* 2. *Specific activities* (*nmol substrate metabolized/min per mg protein in the extract*) *of hepatic enzymes for laying hens given isoenergetic low-linoleate, high-linoleate or conventional diets either* ad lib. *or at a restricted intake* (75–85% ad lib. *intake*)

Expt	Diet*	Feeding regimen	ATP citrate lyase (*EC* 4.1.3.8)	NADP–malate dehydrogenase (L-malate: NADP oxidoreductase; *EC* 1.1.1.40)	NADP–isocitrate dehydrogenase (*threo*-D_s-isocitrate: NADP oxidoreductase (decarboxylating); *EC* 1.1.1.42)	Aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase; *EC* 2.6.1.2)	Alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase; *EC* 2.6.1.1)	Glutamate dehydrogenase (*EC* 1.4.1.2)
1	Low-linoleate	Restricted	8·28	210·8	292·6	549·2	10·69	39·9
	High-linoleate	Restricted	5·91	166·1	248·5	407·4	7·76	27·9
	Low-linoleate	*Ad lib.*	7·24	202·0	342·6	431·4	9·31	42·1
	High-linoleate	*Ad lib.*	4·17	183·8	234·9	343·7	5·78	18·4
	SEM (16 df)		1·45	20·10	32·71	46·55	1·64	5·38
2								
Pre-feeding†	Conventional	Restricted	8·96	227·2	441·4	555·3	8·60	33·6
Post-feeding†	Conventional	Restricted	8·96	232·3	395·9	528·6	10·20	35·9
Pre-feeding†	Conventional	*Ad lib.*	6·74	203·7	387·8	524·3	8·17	36·9
Post-feeding†	Conventional	*Ad lib.*	6·50	176·1	316·3	467·6	8·83	24·2
	SEM (16 df)		1·58	32·72	30·99	45·3	1·26	4·86

* For details of diets, see Table 1.

† Hens were killed post- and pre-feeding, 2·5 and 22·5 h respectively after single daily feed at 11.30 hours.

significant effect in either experiment and the time of killing was also without effect in the second experiment.

The mean specific activities of the hepatic enzymes for both experiments are given in Table 3. Analysis of variance indicated that supplementary linoleate had a significant ($P < 0{\cdot}05$) influence on the specific activity of ICD while the effect of linoleate on the other two lipogenic enzymes, ATP citrate lyase and NADP–malate dehydrogenase was close to significance. On an individual treatment basis, the reduction in the specific activity of ICD resulting from supplementary linoleate was statistically significant ($P < 0{\cdot}05$) for the birds given free access to food. Supplementary linoleate also had a significant influence on the specific activities of aspartate aminotransferase ($P < 0{\cdot}05$) and GDH ($P < 0{\cdot}01$) and the effect on alanine aminotransferase just failed to reach significance. Supplementary linoleate significantly reduced the specific activity of aspartate aminotransferase for the birds fed on restricted intakes ($P < 0{\cdot}05$) and that of GDH for the birds given free access to food ($P < 0{\cdot}01$).

Restricted feeding did not significantly influence the specific activity of any of the enzymes studied in the first experiment. In the second experiment restricted feeding had a significant ($P < 0{\cdot}05$) influence on the specific activity of ICD but did not have any effect on the specific activity of any of the other enzymes. The time of killing had no significant effect on enzyme specific activities in the second experiment.

DISCUSSION

Giving the EFA-deficient diet in the first experiment produced larger livers and increased liver lipid concentrations in our hens. Menge (1967) had previously been unable to find any significant increase in liver size in EFA-deficient hens, but there have been a number of reports of fatty liver development and increased liver size in immature chicks given EFA-deficient diets (Balnave, 1970*a*; Roland & Edwards, 1971). In the present work, restricting the daily food intake did not influence the response to dietary fat compared with *ad lib.* feeding. The fact that daily metabolizable energy (ME) consumption was similar for birds given restricted intakes of diets containing linoleate at both levels indicated that the reported effects were caused by the fat per se and not by variations in ME intake.

The specific activities of the hepatic lipogenic and glutamate-metabolizing enzymes studied were all increased, usually significantly, by giving the EFA-deficient diet and this response was observed with both feeding regimens. This would indicate that feeding with the EFA-deficient diet influenced both hepatic lipid and protein metabolism and that such effects were related more to a lack of dietary fat than to variations in dietary energy intake. The increased liver lipid concentrations found in the EFA-deficient hens are presumably related to the increased hepatic lipogenic enzyme activity. Furthermore, the increased specific activities of the glutamate-metabolizing enzymes, particularly GDH, would suggest that protein synthesis was not inhibited in the livers of the EFA-deficient hens. This would imply that liver fat accumulation was related more to an impairment in the release of lipoprotein from the liver than to a reduced synthesis of apolipoprotein (Fukazawa & Privett, 1972).

Balnave & Pearce (1969) found reductions of 50–65% and 45–80% respectively in the specific activities of hepatic ATP citrate lyase and ICD in two experiments where 45-week-old, EFA-deficient hens were given 20 g supplementary maize oil/kg diet on a restricted intake basis for a period of 10–28 d. In the present work, where hens had been maintained for an extended period on the experimental diets and feeding regimens, there were reductions of 30–40% and 15–30% respectively in the specific activities of hepatic ATP citrate lyase and ICD resulting from maize oil supplementation of the EFA-deficient diet. With both enzymes the maximal decrease was found with the *ad lib.* feeding regimen. The smaller responses of these enzymes to dietary fat under restricted feeding in the present, compared with previous work might be taken to indicate a degree of metabolic adaptation to dietary fat concentration. However, age effects may be important, as Demeyer, Tan & Privett (1974), while finding increases in lipogenesis in epididymal fat cells of rats after 5 weeks on an EFA-deficient diet compared with a safflower-oil-supplemented diet, also found that the differences were considerably reduced after 3–5 months.

Enzyme specific activities of hens fed on the semi-purified diets were similar to those of hens fed on the conventional diet and in neither experiment did the feeding regimen significantly influence enzyme activities. Restricted feeding of laying hens is used as a means of reducing body fat deposition, so that it is interesting to note that the time of killing had no effect on the capacity for lipogenesis as measured by hepatic lipogenic enzyme activity in the second experiment. A possible explanation for the high levels of activity found in the 'pre-feeding' hens with restricted intakes is the presence of a diurnal rhythm in metabolism. Such a diurnal variation in metabolic rate for the fowl has been reported by a number of workers, with the maximal metabolism occurring between approximately 08.00 and 12.00 hours (Balnave, 1974). The report of Chandrabose, Bensadoun & Clifford (1971) also indicates that this possibility exists with regard to at least some avian hepatic enzymes, the maximal effect being observed at 12.00 hours.

The slightly lower specific activities observed in the 'post-feeding' hens given free access to food in the second experiment may also be related to the time of killing. As the hens on the *ad lib.* regimen had free access to food at all times, food consumption would occur early in the morning, with the associated response in metabolism being maximal during the following few hours. The 'pre-feeding' hens were killed at 10.00 hours so that the metabolic response would probably be higher at this time compared with the other group fed *ad lib.* which was killed at 14.00 hours.

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