The effects of dietary protein and non-protein nitrogen on liver glutamate dehydrogenase activity in the chick

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1. Diets containing varying levels of crude protein (CP) in the range 9·5–18·5 % with and without supplements of diammonium citrate (DAC) as a source of non-protein nitrogen (NPN) were given to 160 growing chicks from 2 to 4 weeks of age, and their livers were assayed for glutamate dehydrogenase (GDH) activity.

2. Growth rate and total liver protein were increased by raising the protein level from 9·5 to 18·5 % CP. Chicks receiving 9·5 or 12·5 % CP were heavier when they had also received 1·94 % DAC. At 18·5 % CP the addition of 3·88 % DAC depressed growth.

3. GDH activity per unit liver weight and total GDH activity increased with dietary protein level but there were no consistent responses to DAC supplements. It was concluded that liver GDH activity did not provide a useful index of the utilization of NPN.

The effective utilization of non-protein nitrogen (NPN) for growth has been clearly demonstrated in the chick when included as the sole source of non-essential nitrogen in semi-synthetic diets. However, with diets formulated from normal feed ingredients attempts to show satisfactory utilization of NPN have been mostly unsuccessful (Featherston, 1967). In practical diets it is unlikely that a deficiency of non-essential N would be large and only small growth responses could be expected. Thus, other indications of utilization would be useful.

Reviews by Knox, Auerbach & Lin (1956), Ashida (1963) and Harper (1965) indicate that the activity of many enzymes is altered in response to changes in the diet. Glutamate dehydrogenase (*EC* 1.4.1.2) (GDH) has a key role in amino acid metabolism, is held to be responsible for the incorporation of ammonia N into amino groups, and the concentration of this enzyme in the liver has been shown to respond to differences in dietary protein level in the rat (Murumatsu & Ashida, 1962). However, it has only been studied in the chick in relation to a high level of NPN in a semi-synthetic diet (Lee, McNab, Shannon & Blair, 1970). When used as the sole source of non-essential N, diammonium citrate (DAC) depressed liver GDH activity in relation to both liver weight and liver protein.

A series of trials has been carried out to measure the liver GDH activity of chicks fed on diets based upon natural protein sources, with or without supplementary NPN in the form of DAC. A range of protein levels was used, this being one factor which might be expected to affect GDH activity.

EXPERIMENTAL

Diets and treatments

Diets containing protein at several levels were used, ranging from protein concentrations similar to those found in normal chick rearer diets to comparatively low concentrations. All experimental diets were based on a practical chick starter diet, and the use of a protein-free diluent ensured that the amino acid balance remained constant within any experiment, irrespective of protein level. Variation in the level of crude protein (CP) was achieved by mixing appropriate proportions of the starter diet and the protein-free diluent; the compositions of these are given in Table 1. DAC replaced equal quantities of starch and sucrose when included as a source of NPN.

Table 1. *Constituents* (%) *of the chick starter diet and nitrogen-free diluent for the preparation of the experimental diets*

Chick starter		N-free diluent	
Wheat meal	30·0	Starch	50·0
Barley meal	10·0	Sugar	40·0
Maize meal	25·0	Maize oil	4·0
Middlings	15·0	Limestone	2·5
Fish meal	7·5	$CaHPO_4$	3·0
Meat and bone meal	2·5	T.E. 8*	0·4
Soya-bean meal	10·0	NaCl	0·25
Limestone	1·25		
T.E. 8*	0·225		
NaCl	0·225		
Crude protein (N × 6·25)	20·0		

* Mineral and vitamin supplement purchased from Cooper Nutrition Products Ltd.

In Expt 1 the effects of different protein levels in the range 9·5–18·5% CP were studied; in Expts 2 and 3, diets of varying protein level were each supplemented with 1·94% DAC, which contributed 0·24% N, the equivalent of 1·5% CP (N × 6·25); in Expt 4, supplements of 0, 1·29, 2·59 and 3·88% DAC (equivalent to 0, 1, 2 and 3% CP respectively) were added to the 18·5% CP diet.

Birds

Male chicks of a commercial hybrid laying strain were used. After a preliminary period of about 2 weeks during which they were fed on a conventional chick starter diet, birds of similar body-weight were selected for experiment. A randomized block design was used in each experiment. There were two blocks, each of which consisted of one cage of five birds on each treatment. Food and water were available at all times and both day-length and temperature were controlled. At the end of a 14 d experimental period the birds were weighed and slaughtered by cervical dislocation; their livers were rapidly removed and chilled before homogenization for enzyme assay.

Homogenization and enzyme assay

The chilled livers were rapidly weighed and homogenized for 2 min in 9 vol. of deionized water in a Virtis '45' homogenizer (The Virtis Co. Inc., Gardiner, NY). Homogenates were diluted twenty times with 0·1 M-potassium phosphate buffer (pH 7·4) before being assayed for enzyme activity. GDH activity was assayed by determining the rate of oxidation of reduced nicotinamide-adenine dinucleotide (NADH), measured by the change in extinction at 340 nm, using a Unicam SP 800 spectrophotometer. The procedure was that of Wergedal & Harper (1964) with the exception that the concentration of NADH was increased. Thus the concentrations of reagents in the final reaction mixture were $1{\cdot}5 \times 10^{-4}$ M-NADH, $2{\cdot}5 \times 10^{-1}$ M-NH_4Cl, 5×10^{-3} M-α-ketoglutarate, 1×10^{-4} M-ethylenediamine tetra-acetate, 0·1 M-potassium phosphate buffer (pH 7·4). The protein concentration of homogenates was measured by the method of Lowry, Rosebrough, Farr & Randall (1951).

Preliminary studies indicated that the type of homogenizer employed affected the release of activity, as also did the homogenizing medium. A range of homogenizers of different types was tested but the highest and most consistent activities were always obtained by the use of the Virtis '45' unit with water as the medium. The activity of undiluted homogenates was found to remain stable for at least 4 d when stored at 4°. Potassium phosphate buffer was preferred to sodium phosphate in the reaction mixture since the initial response was not always linear with the latter. The activation with adenosine diphosphate reported by Freedland, Martin & McFarland (1966) in studies of GDH activity with quail tissue has been noted but it was not included in the reagent mixture for routine assays.

Statistical analyses

The analysis of variance for randomized blocks was used. In Expts 2 and 3 the main effects and interaction of the factors (level of protein or DAC in the diet) were tested. Further information was obtained in Expts 1 and 4 by comparing the treatment with least protein or DAC, or most DAC with the other treatments (see footnote to Table 2).

RESULTS

The results for individual treatments in all experiments are given in Table 2 and the results for factorial analysis of Expts 2 and 3 in Table 3.

Increases in the concentration of intact dietary protein from 9·5% to 18·5% CP produced significant increases for live-weight gain ($P < 0{\cdot}01$) in Expt 1, some of this response being due to food intake as the group receiving the lowest protein level had the lowest food intake and the poorest growth. The addition of 1·94% DAC to the diets containing 9·5% and 12·5% CP increased live weight significantly.

In Expt 4, graded levels of DAC added to the high-protein diet caused a depression in growth which was significant for the addition of 3·88% DAC ($P < 0{\cdot}05$).

GDH activities were expressed in several ways in relation to the size of the birds and their livers. In Expt 1 activities per unit liver weight and total GDH activities

Table 2. *Approximate food consumption and live-weight gains of chicks during the 14 d experimental period, and weight, protein content and glutamate dehydrogenase (GDH) activity of their livers at the end of the period*

(Mean values with their standard errors for two groups of five birds/treatment; a significant *F*-test to compare the four treatments is shown by: † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$)

Expt no.	Protein in diet (%)	DAC§ in diet (%)	Food consumed (g/bird)	Live-wt gain (g)	Liver wt: Total (g)	Liver wt: As % body-wt	Liver protein (g)	GDH activity (μmol NADH oxidized/min): per g wet liver	GDH activity (μmol NADH oxidized/min): Total
1	9·5	—	382	73	7·50	4·00	0·97	56·0‡	409
	12·5	—	445	135	9·32	3·75	1·32	66·1	610
	15·5	—	476	178	9·47	3·23	1·45	66·9	616
	18·5	—	498	199	8·74	2·81	1·48	73·0	637
SE				5·8 **	0·55	0·17 *	0·076	3·8	34 *
2	9·5	—	464	105	8·13	3·29	0·91	64·5	526
	9·5	1·94	472	129	8·94	3·30	0·98	65·8	583
	12·5	—	525	153	10·13	3·48	1·18	65·8	668
	12·5	1·94	484	164	9·48	3·12	1·20	83·1	797
SE				3·3 **	0·35 †	0·13	0·033 *	5·9	60
3	13·5	—	427	132	7·58	2·88	1·08	76·4	570
	13·5	1·94	428	140	8·34	3·15	1·21	76·2	613
	18·5	—	436	160	7·86	2·78	1·15	81·9	655
	18·5	1·94	434	172	8·70	2·89	1·29	71·8	621
SE				6·9 †	0·38	0·11	0·057	7·3	32
4	18·5	—	553	198	11·17	3·06	1·59	65·6‡	729
	18·5	1·29	563	187	10·14	2·85	1·56	73·9	752
	18·5	2·59	534	193	9·73	2·69	1·43	73·2	710
	18·5	3·88	543	163‡	8·84	2·68	1·34	75·8	672
SE				7·7	0·71	0·15	0·089	4·7	86

‡ Significantly lower than all other values in the same experiment ($P < 0.05$). § Diammonium citrate.

were significantly different between diets containing 9·5% CP and those containing greater amounts of CP. When supplements of 1·94% DAC were added to diets containing 9·5%, 12·5% (Expt 2) and 13·5% (Expt 3) CP there was a consistent tendency (not sufficiently great to achieve statistical significance) for total GDH activity to increase. In Expt 4, where graded levels of DAC were added to the high-protein diet, activity per unit liver weight was significantly increased by DAC supplements but there was a tendency for higher levels of DAC to be associated with lower total GDH

Table 3. *Main effects of dietary factors for live-weight gain of chicks during the 14 d experimental period and weight, protein content and glutamate dehydrogenase (GDH) activity of their livers at the end of the period*

(Mean values with their standard errors for four groups of five birds/level of protein or DAC§ in diet‡: A significant main effect of a factor is shown by: † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$)

Expt no.	Protein or DAC in diet (%)		Live-wt gain (g)	Liver wt		Liver protein (g)	GDH activity (μmol NADH oxidized/min)	
				Total (g)	As % body-wt		Per g wet weight	Total
2	Protein	9·5	117	8·53	3·29	0·95	65·1	554
		12·5	158 **	9·80 *	3·30	1·19 *	74·4	733 †
	DAC	0	129	9·13	3·38	1·04	65·1	597
		1·94	147 *	9·21	3·21	1·09	74·4	690
SE			2·3	0·25	0·093	0·024	4·2	43
3	Protein	13·5	136	7·97	3·03	1·14	75·3	592
		18·5	166 *	8·28	2·84	1·22	76·9	638
	DAC	0	146	7·72	2·83	1·11	79·2	613
		1·94	156	8·52	3·03	1·25 †	73·0	618
SE			4·9	0·27	0·078	0·040	5·1	22

‡ There were no significant interactions between level of protein or DAC in the diet.
§ Diammonium citrate.

activity, even though this was not sufficiently marked to reach significance. When calculated in relation to liver protein and body-weight, GDH activities were relatively constant within each experiment, regardless of the changes in dietary protein level or the addition of DAC.

In Expt 2 there was a significant increase in liver size with increasing protein level. Otherwise no consistent changes occurred in response to changes in protein level or DAC. Expression of liver weight as percentage of body-weight in Expt 1 showed that a reduction in the relative liver weight resulted from an increase in the dietary protein level. Changes in the total liver protein content were similar to those for live-weight gain and total GDH activity. There were responses to protein level in Expts 1 and 2 and to DAC in Expt 3.

DISCUSSION

The differences in total GDH activity which have been observed with different treatments may all be attributed to differences in body-weight, and it is concluded that GDH activity is not a useful index of ammonia utilization, being less sensitive than growth. At no time did DAC reduce GDH activity, as was found by Lee *et al.* (1970). The diets used in their studies contained crystalline essential amino acids, presumably at minimum levels of requirement, with NPN as the sole source of non-essential N and hence at a high level. In the present studies intact dietary protein sources were used so that non-essential amino acids were present as alternatives to DAC as sources of non-essential N, as they would be in practical diets. Thus, there was an important difference between the two studies. It is clear that high levels of DAC, which promote growth when included in diets based upon synthetic essential amino acids (Shannon, Blair, McNab & Lee, 1970), will depress growth when used in diets based upon intact proteins. Moran, Summers & Pepper (1967) found that 5% DAC significantly depressed growth when added to a diet containing 10% CP; urea had a less marked effect, suggesting the importance of the form in which the NPN is supplied.

At the lowest protein levels 1·94% DAC enhanced growth. Blair & Waring (1969) obtained a growth response in broilers up to 4 weeks of age by the addition of 1·5% diammonium phosphate to diets having 19·5% CP. However, Moran *et al.* (1967) found 2·0% DAC to have no effect. Thus, it would appear that only a narrow range exists between levels of DAC that stimulate growth, intermediate amounts that have no clear effect and amounts that depress growth. Further attention needs to be given to the balance of dietary amino acids, both essential and non-essential. It was found by Featherston, Bird & Harper (1962) that a 25% excess of essential amino acids prevented a response to NPN in the form of DAC or urea even though no other source of non-essential N was provided. It is important, therefore, in attempting to obtain conditions in which there is a response to NPN to eliminate excesses as well as deficiencies of amino acids since either may limit NPN utilization.

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