

## Amino acid uptake in chickens subjected to increasing levels of quantitative food restriction

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1. Three groups of cockerels were reared from 3 to 11 weeks of age on increasingly severe quantitative food restriction treatments, resulting in body-weight values of 12, 19 and 27% below that of a control group which was fed *ab lib*.
2. The rate of uptake of L-arginine, glycine, L-lysine and L-phenylalanine was measured in vitro using intestinal rings, over a 5 min incubation period.
3. Uptake of L-arginine was significantly increased with increasing degrees of body-weight restriction. This amino acid and L-lysine were the only two to show a significantly enhanced uptake rate as a result of the restriction treatments.
4. No significant differences were noted in the case of glycine or L-phenylalanine uptake following food restriction, indicating a certain selectivity in the alteration of absorption rates following food restriction, when such tests are conducted in vitro.

Various theories have been propounded concerning homeostatic mechanisms in the animal which are purported to respond to dietary manipulation. Such responses include a decline in absorption rate of an amino acid following hyperaminoacidaemia, induced by feeding a diet containing an excess of that amino acid (Wapnir, Hawkins & Lifshitz, 1972); and a compensatory mechanism which increases the ability of the small intestine to absorb glucose and L-histidine following varying degrees of dietary restriction (Kershaw, Neame & Wiseman, 1960).

These homeostatic mechanisms appear to be more sensitive to the feeding of diets unbalanced with respect to certain amino acids than to the quantitative restriction of growth either by semi-starvation or by intermittent feeding. Thus, reports concerning enhanced transport of amino acids following the feeding of unbalanced diets are generally in agreement (Wapnir *et al.* 1972; Nakamura, Yasumoto & Mitsuda, 1972; Wapnir & Lifshitz, 1974), whereas those concerning the effect of semi-starvation or intermittent feeding on amino acid absorption are often contradictory (Fabry & Kujalova, 1958; Kujalova & Fabry, 1960; Preston-Mafham & Sykes, 1970).

Also, reports vary according to the method of determination of transport rates. Neame & Wiseman (1959) and Kershaw *et al.* (1960), using in vivo methods, were among the first to demonstrate an enhanced rate of uptake of both glucose and L-histidine following semi-starvation, but in vitro experiments showing that amino acid uptake is enhanced following quantitative food restriction could not be found in the literature.

Because the restricted feeding of pullets during the growing period is now an accepted practice, it is important to understand the effects of such treatments on basic physiological processes such as uptake of amino acid by the intestine. For this reason, such a study was conducted in conjunction with a food restriction experiment in which the growth of pullets was restricted quantitatively (Gous & Stielau, 1976). In the present experiment the effect of three levels of quantitative food restriction on the rate of uptake of four amino acids was studied, using cockerels as experimental material, assuming the effects of dietary restriction on growth and absorption rate to be similar for both males and females.

## EXPERIMENTAL PROCEDURE

*Experimental birds*

Cockerels of a Leghorn  $\times$  Australorp strain were reared from 3 to 11 weeks of age in wire colony cages, six birds per cage. The rearing diet shown in Table 1 was offered. Three replications of four treatments were used, these being: (1) a control treatment, birds being fed *ad lib.*; (2) a quantitative food restriction treatment designed to produce chickens with a body-weight 10% below that of birds fed *ad lib.*; (3) as in (2), but a 20% body-weight restriction was applied; (4) as in (2), but body-weight was restricted by 30% below the body-weight of chickens fed *ad lib.*

In the quantitative restriction treatments, a 2 d quantity of food was supplied every alternate day in order to reduce variability in body-weight within each treatment. The amount of food supplied on the basis of weekly weight measurements of birds in each treatment, was increased or decreased according to the deviation from the target body-weight.

*Preparation of tissue*

All chickens were fasted for 16 to 20 h before experimentation but free access to water was allowed during this period. Had the experiment been conducted *in vivo* it may be argued that acclimatization to this 16–20 h starvation period may have been possible, if changes in uptake rate are mediated in part by the level of amino acids in the blood (Wapnir *et al.*, 1972). However, such changes in *in vitro* uptake rates as may be influenced by the dietary treatments are presumed not to be altered appreciably by starvation within the 16–20 h period.

Chickens were killed by neck dislocation, and approximately 40 cm of intestine immediately anterior to the yolk stalk was excised and rinsed free of digesta with a jet of distilled water. The intestine was then laid on a board covered with filter paper and cut into 1 cm lengths with the aid of an apparatus containing five razor blades spaced at 1 cm intervals. Eight segments were allocated in random order to each of four dishes which then facilitated rapid transfer of these segments to the 25 ml Erlenmeyer flasks in which the incubation took place. These flasks were held at 30° in a shaking water bath, and were continuously aerated with a gas mixture of oxygen and carbon dioxide (95:5, v/v).

*Conditions of incubation*

The method used in this experiment to determine the rate of uptake of amino acids by isolated segments of chicken intestine was essentially similar to that used by Finch & Hird (1960). The incubation medium was sampled at various time intervals, and uptake was calculated from the rate of disappearance of the <sup>14</sup>C-labelled amino acid from this incubation medium.

The tissue was incubated in a medium of Tris buffer (Kimmich, 1970) containing 120 mM-NaCl; 20 mM-Tris-Cl (pH 7.4); 3 mM-K<sub>2</sub>HPO<sub>4</sub>; 1 mM-MgCl<sub>2</sub>; 1 mM-CaCl<sub>2</sub>. The final pH was 7.6. In addition the medium contained 0.3% (w/v) D-glucose.

The four amino acids, L-arginine, glycine, L-lysine and L-phenylalanine, were made up to a concentration of 40 mM with respect to the amino acid, to give a final concentration of 2 mM when this solution was added to the buffer. To each amino acid solution was added the corresponding U-<sup>14</sup>C-labelled amino acid, but at a negligible concentration compared with that of the <sup>12</sup>C-labelled amino acid.

To 10 ml of buffer in each flask, 0.5 ml of amino acid solution was added so that, before killing each chicken, four 25 ml flasks each contained one of the four amino acids to be used in the experiment. A duplicate 0.05 ml sample of this medium was removed from each flask in order to calculate the radioactive content at zero time. Incubation was started

Table 1. *Composition (g/kg) of experimental diet*

Maize	351	Analysis	
Sorghum	160	Crude protein (%)	15.37
Wheat bran	116	ME (MJ/kg)	10.80
Wheat pollard	195	Calcium (%)	0.78
Sunflower meal	47	Phosphorus (available) (%)	0.50
Fish meal	60	Amino acids (calculated) (%)	
Monocalcium phosphate	9	Lysine	0.71
Limestone powder	8	Arginine	0.88
Salt	3	Methionine	0.30
Molasses	50	Cystine	0.55
Vitamins and minerals*	1	Tryptophan	0.18

\* Provides (/kg diet): 25 mg thiamin, 16 mg riboflavin, 20 mg pantothenic acid, 6 mg pyridoxine, 0.6 mg biotin, 4 mg pteroylmonoglutamic acid, 5 mg menaphthone, 0.02 mg cyanocobalamin, 150 mg nicotinic acid, 250 mg ascorbic acid, 4.2 mg  $\alpha$ -tocopherol (25%) 150 mg retinol (1982  $\mu$ g/g), 7.2 mg cholecalciferol (2000  $\mu$ g/g), 2 g choline chloride, 1 g  $\text{KH}_2\text{PO}_4$ , 800 mg NaCl, 50 mg  $\text{Fe}^{2+}$ , 250 mg  $\text{MgSO}_4$ , 20 mg  $\text{MnSO}_4$ , 1 mg KI, 1.28 mg  $\text{CuSO}_4$ , 20 mg  $\text{ZnCO}_3$ .

with a time separation of 15 s between flasks and the medium was sampled with the aid of a micro-pipette at 1 min intervals for 5 min.

Radiochemicals were purchased from the Radiochemical Centre, Amersham, Bucks, England. All radioactive amino acids were uniformly-labelled and had a minimum specific activity of 112 mCi/mmol. Other chemicals used were all of analytical grade. Radioactivity in each sample was determined using a Beckman LS200 Liquid Scintillation Spectrophotometer, and 15 ml of a scintillation solution consisting of 2,5-diphenyloxazole (PPO) (10 g), *p*-bis(*O*-methylseryl)benzene (Bis MSB) (1 g), toluene (1 l) and methanol (1 l).

## RESULTS

The body-weight of birds at 11 weeks of age is shown in Table 2. These values are presented as the mean  $\pm$  SE of the mean of sixteen birds per treatment. Body-weight was reduced by 11.8, 19.8 and 27.3 % respectively below that of the control group, these values being close to the intended restriction levels.

The rate of uptake of L-arginine by intestinal preparations of cockerels was significantly increased with increasing degrees of body-weight restriction. This was not the case with the three other amino acids studied (Table 3).

The least-severe restriction treatment resulted in a significant increase in rate of uptake of L-lysine compared with the control treatment. Mean values of uptake rate in the remaining treatments was greater than that of the control rate, but these differences were not significant.

## DISCUSSION

Few critical experiments appear to have been performed in which the effect of food restriction of chickens on intestinal uptake of amino acids has been studied, although a number of reports have been published on such effects following semi-starvation and fasting of rats. One of the first experiments of this kind was conducted by Kershaw *et al.* (1960), where it was shown that an enhanced rate of disappearance of both glucose and L-histidine from the small intestine of the rat *in vivo* followed a period of semi-starvation. The work on rats is not entirely comparable with the present work, as the original body weight of the rats was reduced by about 20 % by means of semi-starvation over a period of 9 d, whereas in the present experiment all birds had a greater body-weight at the end of the 8 week restriction period than at the beginning, i.e. growth rate was decreased, but not

Table 2. *Body-weight (g) of birds used in the experiment*  
(Results presented as mean  $\pm$  SE of mean for sixteen birds/treatment)

Dietary treatment	Mean body-weight		Body-weight as percentage of control
	Mean	SE	
Control ( <i>ad lib.</i> )	923	20	100
90 % of control body-weight	813	37	88.2
80 % of control body-weight	745	25	80.7
70 % of control body-weight	671	24	72.7

Table 3. *Rate of uptake of four amino acids ( $\mu$ mol) by intestinal rings prepared from cockerels subjected to three levels of quantitative food restriction for 8 weeks*

(Eight 1 cm long rings of small intestine incubated with amino acid at 2 mM concentration, uptake at 5 min taken as a measure of the rate of uptake. Results presented as the mean  $\pm$  SE of mean for 16 replications of each treatment)

Amino acid	Restriction treatment			
	Control ( <i>ad lib.</i> )	90 % of control	80 % of control	70 % of control
L-arginine	9.4 $\pm$ 1.4 <sup>b</sup>	9.8 $\pm$ 1.1 <sup>b</sup>	12.4 $\pm$ 2.2 <sup>ab</sup>	14.6 $\pm$ 1.8 <sup>a</sup>
Glycine	21.3 $\pm$ 1.9 <sup>a</sup>	24.2 $\pm$ 2.4 <sup>a</sup>	20.2 $\pm$ 2.0 <sup>a</sup>	23.7 $\pm$ 1.9 <sup>a</sup>
L-lysine	19.4 $\pm$ 2.1 <sup>b</sup>	26.8 $\pm$ 3.0 <sup>a</sup>	22.5 $\pm$ 2.9 <sup>ab</sup>	22.1 $\pm$ 2.2 <sup>ab</sup>
L-phenylalanine	27.5 $\pm$ 2.7 <sup>a</sup>	31.6 $\pm$ 2.9 <sup>a</sup>	29.1 $\pm$ 2.7 <sup>a</sup>	25.8 $\pm$ 2.6 <sup>a</sup>

a, b Within rows, values with the same superscript do not vary significantly ( $P < 0.05$ ). Differences assessed for significance by applying an analysis of variance followed by individual comparisons between the control and restriction treatments by means of *t*-tests (Rayner, 1967).

reversed. Also, experiments of this nature conducted *in vivo* might be expected to yield results different from those conducted *in vitro*, if uptake regulatory mechanisms (if any) are situated not in the intestine itself, but in other regions of the body.

No other experiment appears to have demonstrated successfully an enhanced rate of uptake following quantitative food restriction. Kujalova & Fabry (1960) could find no increase in absorption of amino acid following a 6 week period of intermittent starvation of rats, although these workers later reported an increase in uptake of both glucose and fat following such treatment (Fabry & Kujalova, 1958). In only one experiment out of three could Preston Mafham and Sykes (1970) demonstrate a significant increase in the rate of absorption of glucose following a reduction of body-weight of chickens over a period of 5 d. Uptake of L-histidine and glucose following an infection of *Eimeria acervulina* were, however, significantly reduced in that experiment.

It has been shown that when an amino acid is fed to rats in excess of normal requirement (Wapnir *et al.* 1972) it causes a decrease in the subsequent absorption rate of that amino acid. Similarly an enhanced absorption of an amino acid has been shown to result following the feeding of a diet low in that amino acid (Nakamura *et al.* 1972; Wapnir & Lifshitz, 1974). However, the uptake of amino acids other than those unbalanced in the diet does not appear to be affected by such treatment (Nakamura *et al.* 1972). These latter workers concluded that the mechanism controlling the rate of uptake of amino acid differs according to the method of growth restriction applied, i.e. the compensatory mechanism that apparently controls rate of uptake of the amino acid used to unbalance a diet is entirely different from that involved in semi-starvation and in protein deficiency. Much of the work that has been reported on the effects of malnourishment on amino acid uptake has been concerned with

unbalanced amino acid diets, and if the mechanisms controlling uptake under these circumstances do differ from those regulating uptake following quantitative restriction, then this anomaly could account in part for much of the confusion concerning the effects of semi-starvation on uptake of amino acids.

In the present experiment, the effect of dietary restriction on rate of uptake of amino acids was not consistent. Only in the cases of L-arginine and L-lysine did quantitative restriction result in a significant increase in rate of uptake of an amino acid compared with the control treatment. Although the rate of uptake of both these amino acids was greater for each level of restriction than was that of the control treatment, the uptake of L-arginine was significantly enhanced only in the most severe restriction treatments, whereas with L-lysine, the only significantly greater rate of uptake, compared with the control, was by intestinal preparations from the least-severely restricted birds.

The fact that the intestinal transport rates of glycine and L-phenylalanine were not altered by any of the restriction treatments confirms the result of Wapnir & Lifshitz (1974) who noted the same lack of response in the rate of uptake of these two amino acids following the feeding of low protein diets. Their experiment was conducted *in vivo*. It appears therefore that there is a certain selectivity in the alteration of absorption rates following food restriction. In addition, Hindmarsh, Kilby, Ross & Wiseman (1967) have shown that intestinal transport activity in response to semi-starvation varies among species of experimental animals, adding further to the difficulty of elucidating the precise mechanisms involved in such homeostatic processes.

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