

(BAE). Ruminants excrete between 200 and 1500 mg BAE/kgW<sup>0.75</sup> per 24 h, while omnivorous, carnivorous and herbivorous non-ruminant species only excrete between 14 and 120 mg BAE/kgW<sup>0.75</sup> per 24 h (Martin, 1969).

The large aromatic acid output of ruminants may arise either as a consequence of metabolism in ruminant tissues or as a result of extensive microbial fermentations occurring in the rumen. Experiments in this laboratory in which sheep have been fasted for periods of up to 10 days have shown that, while urinary benzoic acid excretion falls to between 6 and 10 mg BAE/kgW<sup>0.75</sup> per 24 h after 3 or 4 days starvation, urinary phenylacetic acid excretion does not fall below 15–20 mg BAE/kgW<sup>0.75</sup> per 24 h after 10 days starvation.

Quinic and shikimic acids are intermediates in the synthesis of aromatic compounds in plants and are known to accumulate in some plant species. Asatoor (1965) has shown that the intestinal microflora of man metabolizes these compounds to benzoic acid. The contents of shikimic, quinic and the related chlorogenic acid (3-*o*-caffeoyl-quinic acid) have been determined in perennial ryegrass (S24) harvested at six stages of growth from that of young leaf to mature herbage. Each of these six cuts of grass were given to each of four sheep and the urinary outputs of benzoic and phenylacetic acids (both free and conjugated) determined. Assuming complete metabolism of the precursors (including the caffeoyl moiety of chlorogenic acid) to benzoic acid the urinary output of benzoic acid by sheep consuming 1000 g/24 h of each cut has been calculated to be: in order of successive increase of maturity of the grass cut, 14.0, 13.9, 9.3, 7.1, 3.2 and 2.2 g.

The average observed outputs of urinary benzoic acid by sheep per 1000 g/24 h food consumed, expressed as a percentage of the maximum that could have been expected from the precursors considered, were, in order of increasing herbage maturity, 103, 50, 67, 96, 178 and 246%. The average urinary output of phenylacetic acid (g BAE) per 1000 g food consumed were, in order of increasing herbage maturity, 1.6, 3.3, 1.5, 0.4, 0.2 and 0.3 g. Phenylacetic acid excretion was exponentially related to the intake of apparently digestible crude protein and may arise from microbial fermentation of phenylalanine and tyrosine in the rumen (Scott, Ward & Dawson, 1964).

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**A chick biological assay for available tryptophan.** By E. J. HARWOOD and D. H. SHRIMPTON,\* *Unilever Research Laboratory, Colworth/Welwyn, Sharnbrook, Bedford*

The present work was begun because no published procedure was known to the authors. After a satisfactory method had been developed the unpublished work of

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K. J. Carpenter & J. Atkinson (personal communication) with diets based on oxidized casein was brought to our knowledge.

Combinations of casein, gelatin, zein and maize gave unsatisfactory assays with non-linear response. The diet given in Table 1 has proved a satisfactory basal diet for assay of vegetable and animal proteins when the amounts added provide not more than 0.03% of tryptophan.

The slope-ratio design consists of a zero dose, single and double doses of each substance and of the standard, and a 'supplemented' dose of each substance. The latter consists of a single dose of the substance plus a single dose of the standard, and is used to test for the presence of an inhibitor or for stimulatory action as proposed by Kodicek & Pepper (1948). Four replicates of four chicks are used for each dose level, the assay period being from 10 to 18 days of age.

Table 1. *Percentage composition of basal diet for chick biological assay of available tryptophan*

Maize gluten feed (29% crude protein)	58.3
Gelatin	8.5
Dried whey	2.5
Maize oil	2.0
Mineral mixture (Dean & Scott, 1965)	4.6
Vitamin mixture (Dean & Scott, 1965)	2.0
Choline chloride	0.2
L-lysine hydrochloride	0.36
DL-methionine	0.29
Procaine penicillin	0.01

Made to 100% with test material and a filler consisting of a mixture of fine sawdust, kaolin and maize oil. The sawdust and kaolin were present in 2:1 ratio and the amount of maize oil adjusted so that the filler and test material have the same metabolizable energy content.

The mean values for total and available tryptophan in five samples of groundnut meal and four samples of Norwegian herring meal were respectively 0.98 and 0.97, and 1.15 and 0.76 g/16 g nitrogen. The standard errors of the biological determinations were 0.03 and 0.01 for the groundnut and herring meals respectively.

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**A chick biological assay for available methionine.** By E. J. HARWOOD and D. H. SHRIMPTON,\* *Unilever Research Laboratory, Colworth/Welwyn, Sharnbrook, Bedford*

The method of Miller, Carpenter, Morgan & Boyne (1965) could not be reproduced satisfactorily by us to give a linear response curve. The deviation from linearity was

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