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STUDIES ON THE INTESTINAL DIGESTION OF NITROGEN BY SHEEP FED FORMALIN-TREATED CASEIN DIETS

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SUMMARY

The intestinal digestion of formalin-treated and untreated casein supplements added to a basal diet of dried grass was investigated using sheep equipped with duodenal and ileal re-entrant cannulae.

The treatment of casein with the formalin resulted in all the supplementary N reaching the duodenum. The abomasal (gastric) juices destroyed the formalin protection and 65% of the treated casein-N was subsequently digested within the small intestine; a further 22% disappeared between the ileum and the faeces. With the untreated casein, only half of the supplementary-N reached the duodenum in an undegraded form, 30% of the untreated casein-N disappeared within the small intestine and a further 14% disappeared beyond the ileum.

A substantial amount of the supplementary-N from each form of casein appeared in the urine; however, net retention of treated casein supplementary-N was 36% of that administered in the diet. Net retention of untreated casein nitrogen was only 17%.

Data are also presented which show substantial increases in the quantities of "essential amino acids" absorbed from the small intestine when the treated casein was fed.

Nitrogen metabolism in the ruminant animal is complicated by rumen microbial degradation of dietary protein to peptides and amino acids (Sym, 1938; Annison, 1956; Warner, 1956) which are then largely deaminated to ammonia (El-Shazly, 1952). This ammonia has then to be utilized, along with amino acids and shorter chain peptides, for microbial protein synthesis before the nitrogen passes out of the rumen to the site of host-animal enzyme digestion in the abomasum and small intestine. On poor quality, low nitrogen diets, this process can be advantageous to the animal because the micro-organisms can utilize non-protein-N as well as protein to produce microbial protein which has a relatively high biological value (McNaught *et al.*, 1954). On a good quality, high nitrogen diet, however, a considerable amount of the ammonia produced by deamination can be absorbed from the rumen and passed to the liver where it is converted to urea. Although some of this urea may be recycled back into the rumen via saliva, a large proportion is likely to be excreted into the urine, via the kidneys. Excretion of urinary urea represents a net loss of nitrogen to the animal.

Recent Australian work has shown that production responses — *i.e.*, wool growth and liveweight gain increases, can be obtained by avoiding this rumen degradation of dietary protein, either by abomasal infusion of amino acids and protein, or by treating dietary protein with formalin to protect it from rumen degradation (Ferguson *et al.*, 1967); this work has been reviewed by Henderson *et al.* (1970).

Although the Australian work clearly showed a response to treated casein supplementation and to abomasal infusions of casein and amino acids, little information is available, other than apparent digestibility and net retention data, to detail the fate of the nitrogenous component which passes beyond the rumen with these treatments. The present study using animals fed supplements of treated and untreated casein was therefore designed to provide quantitative data on the disappearance of nitrogen and the specific amino acids from the main site of host-animal absorption of "utilizable nitrogen" — *i.e.*, the small intestine. To carry out this programme, sheep which had been surgically equipped with re-entrant cannulae at the beginning and at the end of the small intestine were fed diets containing supplements of formalin-treated and untreated casein.

EXPERIMENTAL

SHEEP

Romney wether sheep, 1 to 2 years old and weighing 40 to 45 kg, were used for all experiments. Each sheep was surgically equipped with a rumen cannula and with re-entrant cannulae at the duodenum and ileum (for details of cannulae and surgery see Brown *et al.*, 1969). The sheep were kept in metabolism crates and had constant temperature, constant artificial light, free access to water at all times, and their rations delivered by means of a continuous feeding device (Ulyatt, 1967). Chromic oxide-impregnated paper was administered daily to all sheep to allow conversion of observed duodenal and ileal data to mean 24 hr values (MacRae and Armstrong, 1969) and polyethylene glycol was also infused into the rumen of certain sheep used in a concurrent mineral study (Grace, 1970).

Diets

Two experiments were performed. In Exp. 1 900 g dried grass (dry matter 86%, nitrogen 2.6%, gross energy 4.5 kcal/g, digestibility 74%) was fed alone. In Exp. 2 the same diet was supplemented with either 60 g formalin-treated casein (nitrogen 14.6%, 1968 batch prepared by Dairy Research Institute, Palmerston North) or 60 g untreated casein (nitrogen 15.2%). The supplementary diets were fed according to a cross-over design.

Samples

During each experiment, 7-day collections of faeces and urine were made, followed by 24 hr collections of digesta at the duodenum and at the ileum. All samples taken for analysis were stored at -20° C prior to freeze-drying. Urine samples were collected into 50 ml of 30% H_2SO_4 and stored at -20° C prior to analysis. Details of procedures used during 24 hr duodenal and 24 hr ileal collections are reported by MacRae and Armstrong (1969).

Analyses

Freeze-dried feed, faeces, duodenal and ileal samples were analysed for residual moisture (105° C for 24 hr) and total-N (micro-Kjeldahl). Ammonia-N levels in freeze-dried duodenal and ileal contents were measured by the method of Conway (1957). Urine samples were analysed for total N. The duodenal and ileal samples were also analysed for their content of chromium by the atomic absorption method of Williams *et al.* (1962) so that corrections to mean 24 hr values could be made. The amino acid compositions of feed, duodenum, ileum and faeces samples were determined, after hydrolysis with excess 6N HCl at 110° C for 24 hr in sealed tubes, by the method of Spackman *et al.* (1958) using a Beckman Spinco 120 C automatic amino acid analyser.

RESULTS

Total Nitrogen (N)

The quantities of N present in the feed, duodenal, ileal, faecal and urine samples of sheep fed diets of dried grass (D.G.), dried grass plus untreated casein (U.C.) and dried grass plus treated casein (T.C.) are presented in Fig. 1. Duodenal samples contained varying amounts of ammonia-N (present as the chloride — large secretions of HCl in

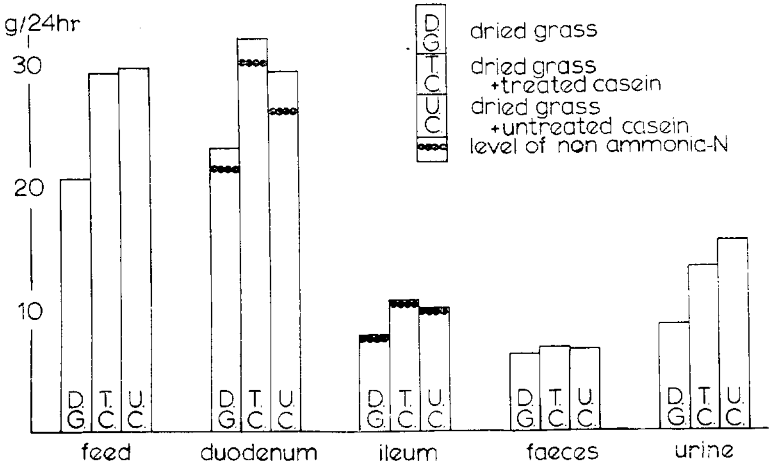

FIG. 1: *Quantities of nitrogen in feed, duodenum, ileum, faeces and urine.*

the abomasum result in a duodenal pH range 2.5 to 3.5), the highest levels of ammonia-N being present in the duodenal contents from the U.C. diet. The absorption of this ammonia within the small intestine is not considered nutritionally important relative to disappearance of other nitrogenous constituents. For this reason, Fig. 1 indicates the quantities of non-ammonia-N (N.A.N.) present in the duodenal and ileal samples. It will be noted that negligible ammonia-N was present in the ileal samples.

With the D.G. and the T.C. diets, the levels of N.A.N. reaching the duodenum were similar to their respective levels of dietary-N. However, on the U.C. diet, the level of N.A.N. reaching the duodenum was less than the level of dietary-N. This suggests that there was some degradation of the U.C. diet anterior to the duodenum which did not occur with the other two diets. The differences between the levels of N.A.N. at the duodenum and at the ileum on the three diets show that disappearance of N.A.N. within the small intestine was in the order T.C. diet > U.C. > D.G. diet.

In the D.G. and the T.C. diets, the main site of disappearance of N.A.N. was within the small intestine; some further disappearance occurred beyond the ileum. However, with the U.C. diet there was a net disappearance of N.A.N. between the feed and the duodenum. This can probably be associated with the increase that was observed in the urinary-N level on the U.C. diet over that for the T.C.

diet. At the same time, however, the large increases in urinary-N for both these diets when compared with the D.G. diet suggest a considerable amount of the extra N.A.N. which disappears beyond the duodenum does eventually leave the animal as urine. Faecal-N levels were similar for all three diets.

If the assumption can be made that the digestion pattern obtained with the D.G. diet would remain constant when the two N supplements were incorporated into the U.C. and T.C. diets, then the D.G. diet values for N and N.A.N. can be subtracted from the corresponding values for the U.C. and the T.C. diets to indicate the fate of the supplementary-N given in the U.C. and T.C. diets. The resulting values, worked out as percentages of the supplementary-N fed, are given in Table 1.

Protection of the casein with formalin allowed virtually all the extra N to reach the duodenum as N.A.N. whereas only half of the untreated casein-N emerged from the rumen, to the site of host animal digestion, as N.A.N. As a result, there was a much higher digestion and disappearance of treated casein-N (65%) than of untreated casein-N (30%) within the small intestine. However, a further 22% of the treated casein-N and 14% of the untreated casein-N disappeared beyond the ileum. The overall apparent digestibilities were treated casein 89% and untreated casein 93%. The net retention of N for the treated casein supplement (36%) was much higher than for the untreated casein supplement (17%); these figures closely reflect the relative differences in disappearance of N.A.N. within the small intestine on the two diets.

TABLE 1: FATE OF SUPPLEMENT-N ADMINISTERED AS TREATED AND UNTREATED CASEIN

	Treated Casein Diet (%)	*Untreated Casein Diet* (%)
Supplementary-N supplied in feed	100	100
Non-ammonia supplementary-N reaching duodenum*	98	54
Non-ammonia supplementary-N reaching ileum*	33	24
Absorption of non-ammonia supplementary-N within small intestine	65	30
Supplementary-N in faeces	11	7
Supplementary-N in urine	53	76
Net retention of supplementary-N	36	17

*Values are total nitrogen minus ammonia-N

AMINO ACIDS

Levels of methionine and leucine measured in the feed, duodenum, ileum and faeces of all three diets are presented in Fig. 2. There was an increase in methionine concentration between the feed and the duodenum, presumably coming from a net rumen synthesis of the amino acid. This increase was paralleled with the T.C. diet, the methionine of the casein being protected from rumen degradation, but with the U.C. diet there was considerable methionine degradation within the rumen which resulted in a duodenal methionine value for the U.C. diet which was intermediate between the D.G. and the T.C. levels. Ileal and faecal levels for all three diets were similar, so it is obvious that the disappearance of methionine within the small intestine was greatest with the T.C. diet and lowest with the D.G. diet.

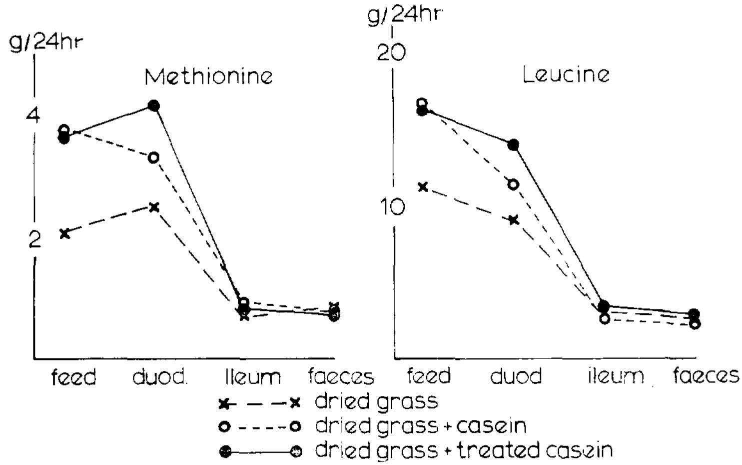

FIG. 2: *Quantities of methionine and leucine passing through the digestive tract.*

Leucine underwent degradation within the rumen on the D.G. diet, resulting in a lower duodenal level; however, the T.C. diet again paralleled this drop, whereas the U.C. diet showed a considerably greater reduction to the duodenum.

Other amino acids which gave net synthesis within the rumen similar to the methionine pattern included the "essential amino acids", isoleucine, lysine, threonine and valine, and the "non-essential amino acids", aspartic acid, glutamic acid and tyrosine. The other common amino

TABLE 2: PERCENTAGE INCREASES IN THE ABSORPTION OF ESSENTIAL AMINO ACIDS WITHIN THE SMALL INTESTINE OVER THE ABSORPTION VALUES OBTAINED WITH DRIED GRASS ALONE, WHEN TREATED AND UNTREATED CASEIN SUPPLEMENTS WERE FED

	Dried Grass plus Treated Casein (%)	*Dried Grass plus Untreated Casein (%)*
Arginine	33	30
Histidine	39	33
Isoleucine	38	33
Leucine	54	29
Lysine	31	21
Methionine	78	33
Phenylalanine	43	16
Threonine	33	8
Valine	37	4

acids, excluding tryptophan and cysteine which were not determined, gave a net degradation within the rumen similar to the leucine pattern.

The percentage increases in disappearance of the "essential amino acids" within the small intestine of sheep fed the U.C. and the T.C. diets over the disappearance when the D.G. diet was fed are given in Table 2. Tryptophan is not considered here because of difficulty in estimation. Arginine, histidine, isoleucine and lysine show little difference between the U.C. and the T.C. diets, but for leucine, methionine, phenylalanine, threonine and valine there were considerably greater increases in the disappearance of each amino acid with the T.C. diet than with the U.C. diet. This was primarily due to considerable degradation of the U.C. diet amino acids before they reached the duodenum. There is also evidence that different amino acids in the treated casein disappeared within the small intestine at different rates.

DISCUSSION

It would appear from the results reported above that the formalin treatment process was extremely efficient in its protection of the casein from rumen microbial degradation. This is borne out by the parallel increases of N and certain amino acids between the feed and the duodenum in the dried grass fed alone and the dried grass plus treated casein diets (see Figs. 1 and 2) while the untreated casein showed a considerable reduction in non-ammonia-N over this region (see Table 1). The levels of N at the ileum show that all three diets were digested to a considerable extent in the small intestine; in the case of the treated casein diet, this indicates that the formalin coating was destroyed to a large extent by the gastric juices of the abomasum.

The ultimate apparent digestibility values for treated and untreated casein, 89% and 93%, respectively (Table 1), are in close agreement with those obtained with Australian prepared casein (Reis and Tunks, 1969 — *i.e.*, 90% and 96%, respectively), but the present work further shows that the whole of this digestion beyond the rumen does not take place within the small intestine. From Table 1, 22% of the treated casein and 17% of the untreated casein supplementary-N disappears beyond the ileum. This is an area of secondary microbial digestion and it is probable that a considerable amount of the digested N within this area enters the blood stream as ammonia which would be quickly converted to urinary-N.

The increased intestinal absorption of methionine on the treated casein diet is of the order which might be expected to give substantial wool growth increases, especially in sheep which are on lower N planes of nutrition than in the present work. Indeed, the actual increased absorption of methionine with the treated casein diet is the same order as that administered by Reis (1967) per abomasum to obtain substantial wool growth increases.

The work of Clarke *et al.* (1966) suggests that, on high N diets such as were fed in the present study, one would expect a duodenal-N level considerably lower than the dietary level, because of microbial degradation of dietary protein within the rumen (when feeding 24.8 ± 1.7 g N they report duodenal-N levels of 19.2 ± 3.5 g). This did not happen in the present work. However, in this work the sheep were fed by means of continuous feeder machines which deliver the rations continuously throughout the 24 hr period rather than in one or two large feeds. This could have a significant effect on the microbial utilization of N within the rumen, a factor which must be borne in mind when assessing the present results for the untreated casein supplement. The supplements were also delivered to the animals on the continuous feeders so the sheep received 2½ g supplement per hour rather than one large intake of 60 g (once daily feeding) or two intakes of 30 g (twice daily feeding). In the case of the untreated casein, this continuous intake would greatly reduce rumen-ammonia production peaks which might be expected with the once or twice daily feeding.

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