Amino acid requirements of South African Mutton Merino lambs 3. Duodenal and whole empty body essential amino acid profile

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The duodenal and whole empty body essential amino acid (EAA) profile of South African Mutton Merino lambs fed a standard growth diet was investigated. The data of 20 lambs were used. With the exception of phenylalanine, significant (p < 0.05) differences between the EAA concentrations of the duodenal digesta and whole empty body occurred. From the chemical score, it was concluded that the duodenal digesta was first-limiting in histidine and second-limiting in methionine, followed by threonine and arginine for the whole empty body growth of South African Mutton Merino ram lambs fed a standard diet high in rumen degradable protein. A calculated essential amino acid composition for the correction of imbalances in duodenal digesta was as follows (%): 11.21 arginine; 14.53 histidine; 8.17 isoleucine; 9.97 leucine; 8.64 lysine; 13.87 methionine; 9.97 phenylalanine; 12.54 threonine and 11.11 valine.

Ondersoek is ingestel na die duodenale en totale leë liggaam essensiële aminosuur profiel van Suid-Afrikaanse Vleismerino ramlammers, wat 'n standaard groeidieet ontvang het. Die data van 20 lammers is gebruik. Met die uitsondering van fenielalanien, het daar betekenisvolle (p < 0.05) verskille in die essensiële aminosuurkonsentrasies van die duodenale inhoud en totale leë liggaam voorgekom. Volgens die chemiese telling was die duodenale-inhoud eerste beperkend in histidien en tweede beperkend in metionien, gevolg deur treonien en arginien vir totale leë liggaamsgroei in Suid-Afrikaanse Vleismerino ramlammers, wat 'n dieet hoog in rumen degradeerbare proteïen ontvang het. Die berekende essensiële aminosuursamestelling om die aminosuur wanbalans in die duodenum reg te stel was soos volg (%): 11.21 arginien; 14.53 histidien; 8.17 isoleusien; 9.97 leusien; 8.64 lisien; 13.87 metionien; 9.97 fenielalanien; 12.54 treonien en 11.11 valien.

Keywords: essential amino acids; whole empty body; duodenum; sheep

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Introduction

Protein metabolism and requirements for ruminants are complex owing to the extensive microbial activity in the rumen which modifies the form and amount of protein reaching the duodenum. Yet, as in non-ruminants, the pattern and levels of essential amino acids (EAA) presented to the small intestine for absorption are rate-limiting factors for tissue synthesis in ruminants (Merchen & Titgemeyer, 1992). Unlike swine or poultry, EAA requirements have not yet been established for growing lambs. In principle, protein metabolism at tissue level is not different between ruminant and non-ruminant species. A simple and effective approach is provided by the ideal protein concept when examining the pattern of dietary amino acids in monogastric animals (Fuller & Chamberlain, 1982).

Stern & Hoover (1979) mentioned that many factors, such as composition of diet, frequency of feeding, dietary intake, rumen dilution rates and availability of nitrogen and energy, affect rumen microbial protein synthesis. Therefore, the amino acid composition of the digesta leaving the rumen, do not reflect that of the ingested diet (Tao *et al.*, 1974; Van der Walt & Meyer, 1988; Matras *et al.*, 1991). It will thus be more accurate to compare the amino acid profile of the duodenal digesta contents, originating from a specific diet, quantitatively and qualitatively, with the amino acid requirements at tissue level to detect the limiting amino acids. Loëst *et al.* (1997) focused on determining growth requirements of the South African Mutton Merino lamb by assay of the carcass which was responsible for 57% of the live weight gain. Ferreira *et al.* (1999) found that 47.77 % of the incremental N deposition occurred in the carcass compared with 41.02% in the skin and wool of lambs growing from 30 to 45 kg live weight. Therefore, as far as net amino acid requirements are concerned, the major sites of protein deposition seem to be in the carcass as well as the skin and wool. The concentration (g AA/100 g crude protein) of arginine, histidine, methionine, threonine and valine also differ significantly ($p < 0.05$) between the carcass and whole empty body (Ferreira *et al.*, 1999). These results suggest that the carcass was not representative of the ideal protein needs of growing Mutton Merino lambs, as in the case of pigs.

The purpose of this study was to compare the EAA profile of the whole empty body calculated by Ferreira *et al.* (1999) according to the ideal protein concept, with that of the duodenal digesta content, to detect imbalances for optimal growth when South African Mutton Merino ram lambs were fed a standard growth diet, high in rumen degradable protein (RDP).

Material and methods

The experimental design, material (lambs, diet), sampling procedure, analyses (chemical, statistical) and calculations used were identical to those used in previous studies (Loëst *et al.*, 1997; Loëst *et al.*, 1999; Ferreira *et al.*, 1999).

Results and discussion

Amino acid composition

Table 1 lists the EAA composition of the standard diet, duodenal digesta and whole empty body (g AA/100 g crude protein). The large differences between the EAA composition of the standard diet and duodenal digesta were discussed by Loëst *et al.* (1999). According to Loëst *et al.* (1999) the protein fraction of the duodenal digesta in this study can be expected to be predominantly of microbial origin. Therefore, the large differences between the average EAA profile of microbial protein in the literature and duodenal digesta were unexpected. The mean microbial protein values in the literature seem to contain higher concentrations of individual amino acids such as isoleucine, lysine, methionine, threonine and valine, and a lower arginine concentration than that of duodenal digesta in the present study. However, the microbial protein composition is not a constant value. Stern & Hoover (1979) mentioned that many factors, such as composition of diet, frequency of feeding, dietary intake, rumen dilution rates, availability of sulphur for methionine and cystine synthesis affect rumen microbial protein synthesis. This probably explains the differences in the duodenal amino acid composition and that of mean microbial values reported in the literature. Meissner & Du Plessis (1992) mentioned that the typical escape proteins in plant proteins used in feedlot diets in South Africa are unlikely to alter amino acid passage to the duodenum. According to Storm & Ørskov (1984) the duodenal protein may be deficient for growing lambs in certain amino acids such as methionine, lysine, histidine and arginine because the microbial protein is deficient in these

Table 1 Essential amino acid content of standard diet, duodenal digesta, whole empty body and microbial protein (g AA/ 100 g crude protein)

EAAs	Standard diet	Duodenal digesta[1]	Whole empty body[1]	Microbial protein[2]
Arg	4.96	6.26b	7.72a	5.3
SD		± 1.03	± 0.58	
His	1.97	2.10[b]	4.54[a]	2.1
SD		± 0.23	± 0.40	
Iso	2.85	3.44[a]	3.06[b]	5.8
SD		± 0.71	± 0.33	
Leu	8.29	8.04[b]	8.53[a]	8.0
SD		± 0.82	± 0.64	
Lys	5.23	7.03[a]	6.46[b]	9.2
SD		± 0.99	± 0.46	
Met	1.29	1.91[b]	3.56[a]	2.5
SD		± 0.26	± 0.22	
Phe	6.45	4.98[a]	5.22[a]	5.3
SD		± 0.53	± 0.44	
Thr	3.19	3.11[b]	4.65[a]	5.7
SD		± 0.34	± 0.48	
Val	4.62	4.25[b]	5.18[a]	5.8
SD		± 0.52	± 0.41	

[1] Mean values determined from the 20 South African Mutton Merino lambs used in the present study; [2] data from Chen & Ørskov (1994); [a,b] Values in rows bearing different superscripts are significantly ($p < 0.05$) different.

amino acids.

Loëst *et al.* (1999) cited from the literature that the tissue amino acid patterns are closer related to those needed by the animals than the plasma amino acid patterns, and that plasma free amino acid levels are frequently difficult to interpret because of the multiplicity of factors that can be involved. An exception is the branched chain amino acids (leucine, isoleucine, valine) which are metabolized to only a limited extent in the liver and thus their concentration in the extra hepatic blood circulation reflects protein uptake from the gut (Harper *et al.*, 1984). In order to identify the limiting amino acids for growth, a comparison of the EAA composition of the lambs' whole empty body (ideal protein) and duodenal digesta could be informative.

From Table 1 it is apparent that significantly ($p < 0.05$) lower concentrations of arginine, histidine, leucine, methionine, threonine and valine occurred in the duodenal digesta content, when compared to that of the whole empty body. On the other hand the isoleucine and lysine concentrations were significantly ($p < 0.05$) higher, while phenylalanine was quite similar ($p > 0.05$). From this comparison a first- and second-limiting amino acid can hardly be identified, and thus for a better evaluation, the chemical score and resulting EAA index (Table 2) were calculated according to the method described by Loëst *et al.* (1999). The chemical score presents the proportion of a specific EAA relative to that in whole empty body protein, while the EAA index represents the propor-

Table 2 Chemical score, essential amino acid index and essential amino acids expressed as a percentage of lysine

	Chemical score[1]		EAA: Lysine[2]		
EAA	Standard diet	Duodenal digesta	Standard diet	Duodenal digesta	Whole body
Arg	64	82 ± 15	95	89 ± 3	120 ± 3
His	43	47 ± 7	38	30 ± 6	70 ± 2
Iso	93	114 ± 30	54	49 ± 2	47 ± 4
Leu	97	95 ± 12	159	114 ± 3	132 ± 3
Lys	81	109 ± 17	100	100 ± 0	100 ± 0
Met	36	54 ± 8	25	27 ± 4	55 ± 2
Phe	124	95 ± 15	123	71 ± 5	81 ± 3
Thr	69	68 ± 10	61	44 ± 6	72 ± 4
Val	89	83 ± 14	88	60 ± 3	80 ± 5
EEA (Index)[3]	75	80			

[1] Chemical score presents the proportion of a specific essential amino acid relative to that of whole empty body protein; [2] EAA: lysine expresses each essential amino acid as a percentage of lysine (lysine = 100%); [3] EAA (Index) presents the proportion of all the essential amino acids studied relative to that of whole empty body protein.

tion of the nine EAA's relative to that of whole empty body protein. The influence of microbial intervention is noticeable when the chemical score of the diet and duodenal digesta is compared (Table 2). The chemical scores suggest that the duodenal digesta is first-limiting in histidine and second-limiting in methionine followed by threonine and arginine. A more than adequate ratio was present for only isoleucine and lysine.

It was suspected, according to the literature discussed by Loëst *et al.* (1999), that methionine would be first- or second-limiting when focused on growth requirements by assay of the whole empty body. Loëst *et al.* (1999) found methionine to be the fourth-limiting in the South African Mutton Merino lamb while isoleucine, leucine and phenylalanine appeared to be in excess for the carcass growth of lambs. This is most likely due to the fact that the study focused on growth requirements by assay of the carcass. The skin and wool amino acid profile was not considered. Nimrick *et al.* (1970), Owens *et al.* (1973) and Storm & Ørskov (1984) found lysine to be second-limiting for growing lambs. This finding could not be confirmed in the present studies because the lysine content of the duodenal digesta and carcass investigated by Loëst *et al.* (1999) were quite similar ($p > 0.05$) while a significantly ($p < 0.05$) higher duodenal than whole empty body lysine content was calculated in the present study (Table 1).

Evidence that the duodenal digesta has provided an imbalanced ratio of EAAs for absorption was found in the chemical score of the present study (Table 2). The results from the present study indicated that histidine, methionine, threonine and arginine would appear to be limiting for whole empty body growth of lambs fed a standard diet with a high degradable protein (± 80%) content. A summary of results in the literature (Nimrick *et al.*, 1970, Owens *et al.*, 1973, Storm & Ørskov, 1984; Chen & Ørskov, 1994) also indicated that these amino acids have previously been reported to be limiting for growing lambs. The other two limiting amino acids in the literature (lysine; valine) however, were not limiting in the present study. These differences were most likely due to different diets and species used during the trails, as well as different methods applied to assess requirements.

Amino acid imbalances

In order to provide a direct comparison of the EAA composition regardless of protein quantity, the values for duodenal digesta and whole empty body were expressed as a percentage of lysine (Table 2). According to Loëst *et al.* (1999) emphasis can be placed on establishing accurate lysine requirements under various circumstances, after which the remaining EAA requirements can be calculated. Loëst *et al.* (1999) also quote the three primary reasons why lysine is selected as the reference amino acid. From the EAA to lysine ratios shown in Table 2 it is interesting to note that except for isoleucine in the duodenum, all the other ratios exhibit lower values when compared to that of the whole empty body. These differences are quite substantial and it can thus be concluded that the EAA patterns of the duodenal digesta are disproportionate to those of the whole empty body. Several studies (Storm & Ørskov, 1984; Nimrick *et al.*, 1970; Owens *et al.*, 1973) have focused on limiting amino acids and ignored EAAs which may have occurred in excess and possibly caused a depression in amino acid and/or other nutrient utilization.

According to the EAA to lysine ratios (Table 2), it can be noted that leucine and phenylalanine concentrations in particular, are exceptionally high in the diet when compared to those of the whole empty body. Similar to the findings of Loëst *et al.* (1999), it was evident that during the present study, the micro-organisms in the rumen of the lambs were capable of modifying the standard diet protein to a more favourable protein with more appropriate EAA patterns. In the present study, the imbalances of specific EAAs in the duodenal digesta relative to those of whole empty body protein (chemical score) could possibly be corrected by following one of two strategies: if the amino acids in excess, according to the chemical score, are quantitatively oversupplied to the duodenum, the infusion of amino acids that appear to be limiting according to the chemical score, may be the obvious strategy to follow. By supplementing the amino acids in short supply, the amino acids in excess will decrease in proportion to those that were limiting. However, if those EAAs in excess are quantitatively undersupplied in the duodenum, the infusion of a mixture of amino acids at different levels in the duodenum could be an alternative strategy.

The EAA composition of this mixture, to provide the optimal balance required in the duodenum for whole empty body growth, can be calculated from the chemical score obtained for duodenal digesta in the present study by using the following calculations.

$Y_i = (100 - X_i) + 100$
where
X_i = chemical score for an individual essential amino acid in duodenal digesta
Y_i = units of an individual essential amino acid as a chemical score relative to whole empty body protein and
$Z_i = Y_i \times 100 / \Sigma Y_{i \ldots 9}$
where
Z_i = percentage of each individual amino acid in the mixture.

According to these calculations, the EAA mixture listed in Table 3 can be provided to the duodenal digesta at different levels to correct imbalances occurring when South African Mutton Merino ram lambs are fed the standard diet used during the present study. The level where optimal supply of amino acids occur can be evaluated by means of a nitrogen balance study, plasma amino acid concentration and growth studies.

Based on research with other species and on a few ruminant experiments, differences probably exist in efficiency of absorption and utilization of amino acids (Polan, 1992). The possible influence of these factors as well as conversion of amino acids in the liver and in the duodenum also need further investigation. For instance, Harper & Rogers (1965) found in rats that surplus amino

Table 3 Calculated essential amino acid composition for the correction of imbalances in duodenal digesta

EAA	Chemical score: Duodenal digesta[1]	Chemical score: Supplementary mixture[2]	Mixture[3] %
Arg	$82^{(Xi)} \pm 15$	$118^{(Yi)}$	$11.21^{(Zi)}$
His	47 ± 7	153	14.53
Iso	114 ± 30	86	8.17
Leu	95 ± 12	105	9.97
Lys	109 ± 17	19	8.64
Met	54 ± 8	146	13.87
Phe	95 ± 15	105	9.97
Thr	68 ± 10	132	12.54
Val	83 ± 14	117	11.11

[1] Chemical score for an individual essential amino acid relative to that of whole empty body protein: Xi; [2] Units of an individual essential amino acid as a chemical score relative to whole empty body protein: Yi = (100-Xi) + 100; [3] Percentage of each individual amino acid in the mixture:

$Zi = Yi \times 100 / \Sigma Yi \ldots 9$

acids arriving in the portal circulation after consumption of an imbalanced diet stimulate synthesis or suppress breakdown of protein in the liver leading to greater retention of the limiting amino acid relative to that in control groups. The supply of the limiting amino acids for peripheral tissues such as muscle is thereby reduced. Eventually the free amino acid patterns of both muscle and plasma become so unbalanced as to invoke intervention by the appetite-regulating system to reduce food intake. Growth is reduced as a consequence of the depressed appetite and intake of nutrients. This hypothesis is still accepted as a satisfactory explanation for the effects of amino acid imbalance in the rat (Leung & Rogers, 1987; Tackman *et al.*, 1990) and is thought to have wider application to other mammalian species (Boorman, 1979).

Conclusions

The amino acid composition of the duodenal digesta determined in the present study, differs substantially from that of the mean microbial values in the literature. These differences occurred in spite of the fact that a high degradable protein (± 80%) diet was fed. Accordingly it was suspected that the duodenal digesta would be predominantly of microbial origin. However, it seems as if other factors also affect rumen microbial protein synthesis. Therefore the mean microbial amino acid values in the literature do not seem to be representative of the amino acids available in the duodenum when a high degradable protein diet is fed. It is probably more accurate to compare the amino acid profile of the duodenal digesta content originating from a specific standard diet and type of animal with that of the whole empty body to detect the limiting amino acids. According to the results of the present study the amino acid composition of the duodenal digesta differs significantly ($p < 0.05$), except for phenylalanine, from that of the whole empty body.

Results from the present study pointed out that, according to the ideal protein concept, the duodenal digesta is first-limiting in histidine and second-limiting in methionine followed by threonine and arginine for the whole empty body growth of lambs fed a high degradable (± 80%) standard diet. Based on the chemical score and EAA to lysine ratio the EAA patterns of the duodenal digesta were disproportionate to those of the whole empty body. In this regard D'Mello (1994) suggested that surplus amino acids, causing an imbalance, may stimulate catabolic pathways resulting in the degradation of all amino acids with the inadvertent loss of the limiting amino acid. In the present study isoleucine and lysine appeared to be in excess for the whole empty body growth of lambs. According to D'Mello (1994) an excess of supplementary isoleucine impaired growth performance in chicks. The effects of imbalances in EAAs on the performance of ruminant animals thus demand further investigation.

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