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Investigation of the postruminal methionine requirement of growing lambs by using the indicator amino acid oxidation technique

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ABSTRACT

Methionine was demonstrated to be the most limiting amino acid for growing sheep when microbial protein is the predominant source of absorbable AA, but its specific requirement was not well quantified. The indicator amino acid oxidation (IAAO) method is based on the concept that when the most limiting amino acid (LAA) is deficient for protein synthesis, then all other AAs will be oxidized. With increasing intakes of the LAA, IAAO will decrease until the requirement for the LAA is met, and there is no further change in the indicator oxidation afterwards. Therefore, the catabolism of ^{13}C-Phe was used to indicate the effects of varying duodenal infusing levels of Met on the oxidation rate by 4-month-old Kazakh lambs (30.8 ± 0.4 kg BW). After a 6-d adaptation period, the IAAO test was conducted on d 7. Each lamb received a priming dose of 0.67 mg/kg BW of ^{13}C-Phe injected *via* the jugular vein, together with 0.18 mg/kg BW of $NaH^{13}CO_3$ at time 0. Then, constant infusions [1.33 mg ^{13}C-Phe/(kg h)$^{-1}$] were started. Breath samples were collected prior to and half-hourly between 90 and 210 min after the start of the ^{13}C-Phe infusions. An estimate of the mean Met requirement was determined by a breakpoint analysis of the rate of $^{13}CO_2$ released by ^{13}C-Phe oxidation, with a 2-phase linear regression model. The oxidation rate of ^{13}C-Phe decreased ($P < 0.001$) with increasing amounts of methionine infused in a dose-dependent fashion up to 1.65 g/day, and it was not different from 1.65 to 2.15 g/day ($P > 0.05$). The mean Met requirement was estimated to be 1.28 ± 0.11 g/day of supplementation. The total postruminal minimum requirement of Met would be 2.02 g/day when the basal passage of Met (0.74 g/day) was summed.

1. Introduction

It is well documented that methionine (Met) and cysteine (Cys) are the primary limiting amino acids for wool production in sheep (Liu and Masters, 2003). It was also demonstrated that wool production and live-weight gain can be improved by supplementing sheep with Met (Williams et al., 1988; Reis et al., 1990; Mata et al., 1995). However, the specific requirement of Met for growing ruminants remains elusive (MacRae et al., 1997). Traditionally, amino acid (AA) requirements for growing animals were quantitatively estimated by feeding diets of varied AA contents and measuring the growth rate, nitrogen (N) retention, feed efficiency and plasma AA concentrations. For ruminants, pregastric metabolism necessitates the quantification of post-ruminal

Abbreviations: AA, amino acids; N, nitrogen; IAAO, indicator AA oxidation; DMI, dry matter intake; NDF, neutral detergent fiber; ADF, acid detergent fiber; BW, body weight; TMR, totally mixed ration; APE, atom percent excess

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Table 1
Composition and nutrient levels of three total mixed rations (DM basis).

Ingredients, g/kg of diet	Composition
Ground corn	8.4
Commercial concentrate supplement[a]	35.4
Alfalfa meal	14.4
Wheat straw	28.2
Cottonseed hull	13.6
Total	100.0
Digestible energy, DE[b] (MJ/kg)	11.72
Total digestible nutrients, TDN[b] (%)	64.94
Crude protein, CP (%)	13.00
Neutral detergent fiber, NDF (%)	47.74
Acid detergent fiber, ADF (%)	24.64
Calcium	0.55
Phosphorus	0.34
Concentrate forage ratio	59:41
Arginine	3.73
Histidine	2.78
Isoleucine	2.87
Leucine	6.99
Lysine	3.49
Methionine	1.41
Phenylalanine	3.83
Threonine	3.02
Tryptophan	0.50
Valine	3.40
TEAA[c]	32.02
Alanine	4.79
Aspartic acid	6.72
Cystine	1.76
Glutamic acid	12.92
Glycine	3.06
Proline	4.32
Serine	3.19
Tyrosine	2.01
TNEAA[c]	38.78

[a] The commercial concentrate supplement provided the following per kg of diets: ground corn, 644.6 g;, sprayed corn bran, 42.4 g; DDGS, 60.0 g; soybean meal, 176.9 g; cottonseed protein, 50.0 g; calcium hydrophosphate, 6.2 g; fine limestone, 11.8 g; salt, 5.1 g; vitamin premix, 1.0 g; and trace element premix, 2.0 g.
[b] DE and TDN were calculated values, while the others were measured values.
[c] TEAA stands for total essential amino acids and TNEAA stands for total non-essential amino acids.

requirement of amino acids. Unfortunately, due to the lack of data on the AA composition of endogenous N in sheep, as well as the utilization efficiencies of individual amino acids for various productive purposes, it is very difficult to adapt and use the conventional metabolizable protein requirement system for amino acids (Liu and Masters, 2000). Therefore, an alternative approach is necessary to directly estimate the requirement of individual amino acid for sheep.

The indicator AA oxidation (IAAO) technique, initially developed in pigs (Kim and Bayley, 1983; Ball and Bayley, 1984), has been widely used to determine the AA requirements in pigs (Bertolo et al., 2005; Moehn et al., 2008) and humans (Pencharz and Ball, 2003; Elango et al., 2008). The IAAO allows measurements to be made after a short adaptation period of 2 d for pigs (Moehn et al., 2004) and 2 h for humans (Bross et al., 1998) so that sufficient levels of AA intake can be tested within short experiment durations without changing their physiological state. Therefore, the objectives of this experiment were to determine the supplemental postruminal need for Met by growing lambs and to estimate total postruminal Met minimum requirement of individual lambs under practical feeding conditions.

2. Materials and methods

2.1. Animals and diets

All of the procedures used in this study were approved by the Animal Care and Use Committee of Shihezi University. The procedures and diets used in this study were described in detail by Gao et al. (2016). In brief, three Kazakh lambs (30.8 ± 0.4 kg initial BW at 4-months), each fitted with a permanent cannula in the proximal duodenum, were maintained in individual crates (1.4 × 0.6 m^2) at a constant room temperature of 20 °C under continuous lighting. The lambs were fed twice daily (08:00 and 20:00) with equal parts of the diet in 12-h intervals in order to maintain a relatively stable rumen environment. The feed composition and