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# Effect of feed intake on metabolizable protein supply in Dorper $\times$ thin-tailed Han crossbred lambs



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#### ABSTRACT

We investigated the metabolizable protein (MP) supply in lambs at different levels of feed intake. Twelve Dorper × thin-tailed Han crossbred ram lambs ( $41.3 \pm 2.8$  kg body weight) fitted with ruminal and duodenal cannulae were randomly assigned to one of three levels (n = 4 lambs each) of dry matter intake: *ad libitum* (AL) intake and 70% or 50% of AL intake. Digesta flow was measured using a dual-marker system (Yb and Co). A lower duodenal flow of rumen undegraded nitrogen (RUN) was measured for the 50% AL group (P < 0.05) compared with the other two groups. For lambs of the AL group, the ratio of microbial N/duodenal non-ammonia nitrogen (NAN) was lower (P < 0.05), and the ratio of RUN/duodenal NAN was higher (P < 0.05) compared with the other two groups. The ratio of RUN/N intake was higher for the 70% AL and 50% AL groups compared with that for the AL group (P < 0.05). Apparent post-ruminal N digestibility increased with decreasing feed intake (P < 0.05). A linear correlation was established to predict MP supply (g/day) from the intake of organic matter (kg/day) or crude protein (g/day): MP =  $0.036 (\pm 0.004) \times$  organic matter intake + 50.47 ( $\pm 4.43$ ),  $R^2 = 0.89$ ; MP =  $0.27 (\pm 0.033) \times$  crude protein intake + 49.88 ( $\pm 4.93$ ),  $R^2 = 0.87$ . The current results provide preliminary data of MP requirements for growth of Dorper crossbred lambs.

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#### 1. Introduction

Protein is the most expensive feed ingredient for livestock, and a clear understanding of the protein requirements of animals can be valuable for reducing costs and ensuring farm profitability. The crude protein (CP) system does not differentiate the requirements of ruminal microbes and the host animal. Furthermore, the CP system is based on an invalid assumption that the proteins in all feedstuffs are equally degraded in the rumen, with CP being converted to metabolizable protein (MP) with equal efficiency in all diets (NRC, 2000). Therefore, the requirements based on the CP system do not necessarily reflect the requirements of ruminants and may lead to protein deficiency.

The current nutritional systems for sheep specify protein requirements as MP, including ruminally synthesized microbial CP, rumen undegraded protein, and a smaller proportion of endogenous CP, which contributes to the protein that is digestible in the small intestine (CSIRO, 2007; AFRC, 1993; NRC, 2007). Although

http://dx.doi.org/10.1016/j.smallrumres.2015.10.016 0921-4488/© 2015 Elsevier B.V. All rights reserved. MP may accurately reflect the utilization of protein by ruminants, evaluating MP *in vivo* is not easy because cannulated animals are required and surgical operations are laborious and expensive. Therefore, few *in vivo* studies have been conducted, and *in vitro* or *in situ* protein evaluation systems have been used *in vivo* measurements. However, *in vitro* results do not always accurately reflect the situation *in vivo*.

Thus, we evaluated the MP supply *in vivo* using different levels of feed intake. Previously, our research team systematically investigated the requirements of energy (Deng et al., 2012, 2014; Xu et al., 2015) and minerals (Ji et al., 2013, 2014) in Dorper × thin-tailed Han crossbred sheep, a dominant breed for lamb meat production in China. The present study provides preliminary data for evaluation of MP requirements of these crossbred lambs.

#### 2. Materials and methods

#### 2.1. Animals and diets

This study was conducted at the Experimental Station of the Chinese Academy of Agricultural Sciences (CAAS), Beijing, China from August 2010 to October 2010. The experimental procedures were

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#### Table 1

Ingredients and chemical composition of the experimental diet.

Item	Composition (g/kg DM)
Ingredients <sup>a</sup> (g/kg DM)	
Chinese wild rye hay	553
Corn	292
Soybean meal	138
Calcium carbonate	9.5
Salt	5.6
Mineral/vitamin premix <sup>b</sup>	1.8
Chemical composition (g/kg DM, determined)	
DM (g/kg, as fed)	906
OM	916
CP	112
NDF	380
ADF	241

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.

<sup>a</sup>All ingredients were pelleted (6.0 mm in diameter).

<sup>b</sup>The premix contained (per kg): 22.1 g Fe, 13.0 g Cu, 30.2 g Mn, 77.2 g Zn, 19.2 g Se, 53.5 g I, 9.10 g Co, 56.0 g vitamin A, 18.0 g vitamin  $D_3$ , and 170 g vitamin E.

approved by the Animal Ethics Committee of CAAS, and humane animal care and handling procedures were followed throughout the study.

The trial was conducted for 25 days (Ma et al., 2013). Briefly, twelve 6-month-old Dorper  $\times$  thin-tailed Han crossbred ram lambs (41.3 ± 2.8 kg body weight) were fitted with ruminal and duodenal cannulae and were randomly assigned to one of three levels of dry matter (DM) intake (4 lambs/group) according to a completely randomized design: *ad libitum* (AL), or restricted to 70% or 50% of the AL intake. The diet (Table 1) was offered as a singlepellet mixture (6.0 mm diameter) and fed once daily at 08:00 h. The lambs had free access to clean water at all times. After adaption to dietary treatments for 7 days, lambs were then moved into individual metabolism crates for 12 days including 7 additional days of adaption and 5 days of a digestibility trial. Then, duodenal and ruminal digesta were sampled daily at time hours on 6 consecutive days (Ma et al., 2013). All lambs were weighed at the beginning and end of the digestibility trial.

The elements Yb and Co were used as markers to determine the duodenal digesta flow, and  $^{15}$ N was used as an external microbial marker (Ma et al., 2013). From days 8 to 25, the lambs were fed the experimental diet. Prior to offering the labeled feed, a priming dose that contained half the daily marker intake was administered through the rumen cannula of each animal.

#### 2.2. Measurements and sample collection

#### 2.2.1. Feed, orts, and feces

In the digestibility trial, feces were collected daily at time hours from days 15 to 20. Feces were weighed daily, and then a sample of 10% was collected and pooled across days for each animal, dried at 65 °C, and ground through a 1-mm sieve for analysis. Samples of feed were also collected daily, combined, dried at 65 °C for 72 h, and ground through a 1-mm sieve. Feed refusals were weighed, sampled, dried, ground, and combined for each lamb before analysis.

#### 2.2.2. Duodenal and ruminal digesta collection

From days 21 to 23, a 100-ml sample of duodenal digesta was collected every 6 h, moving the collection time forward 2 h each day to obtain the samples at 2-h intervals. The samples were combined for each lamb and separated into particulate and liquid fractions for analysis of nutrient concentrations and markers (Ma et al., 2013).

Samples of ruminal digesta were collected at 6-h intervals on days 24 and 25 for determination of microbial N yield (08:00, 14:00,

and 20:00 h on day 24; and 02:00, 05:00, 11:00, 17:00, and 23:00 h on day 25; Ma et al., 2013).

#### 2.2.3. Chemical analysis

Feed and orts were analyzed for DM, organic matter (OM), and N. DM was determined by drying samples in an oven at 135 °C for 2 h (method 930.15; AOAC, 1995). OM was measured as the difference between DM and ash content (g/kg DM) and the ash content was measured by placing the samples into a muffle furnace at 600 °C for 6 h (method 924.05; AOAC, 1990). Nitrogen was determined by the Kjeldahl method using Se as a catalyst (Marshall and Walker, 1978), and CP was calculated as  $6.25 \times N$ . The isotopic abundance of <sup>15</sup>N in bacterial N and duodenal fractions was determined by isotope ratio mass spectrometry (Finnigan Mat 251, Thermo Fisher Scientific Inc., USA). The concentrations of Yb and Co in both feed and digesta were separately determined by inductively coupled plasma emission spectrometry (X series 2 ICP-MS, Thermo Fisher Scientific Inc., USA).

#### 2.3. Calculations and statistical analysis

The duodenal flows of nutrients were determined by reconstitution of the duodenal digesta based on Yb and Co concentrations and the content of the nutrients in the particulate and whole fractions (Faichney, 1975). Endogenous N was calculated as 0.10 of duodenal N flow according to the NRC (1985), and rumen undegraded nitrogen (RUN) was calculated by subtracting microbial N and endogenous N from duodenal non-ammonia nitrogen (NAN). Apparent post-ruminal N digestibility was calculated as the disappearance of N (%) as the digesta N flowed from the duodenum to the anus. The MP was calculated as duodenal NAN  $\times$  apparent post-ruminal N digestibility.

The data were analyzed as a completely randomized design using SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA). All results were analyzed using PROC GLM, and the comparison of the means was performed using the least squares means option of SAS. Besides, all data were analyzed by a regression including the linear and quadratic effects of level of intake. Besides, the linear regressions analyses were conducted with PROC REG Statistical significance was accepted if P < 0.05.

#### 3. Results

Lesser duodenal flow of RUN was observed for the 50% AL group (P < 0.05; Table 2). The ratio of microbial N/duodenal NAN was lower (P < 0.05), whereas the ratio of RUN/duodenal NAN was higher (P < 0.05) in lambs in the AL group compared with the other two groups. The ratio of RUN/N intake was higher for the 70% and 50% AL groups compared with that of the AL group (P < 0.05).

Apparent post-ruminal N digestibility increased with decreasing feed intake (P < 0.05; Table 3). Both MP/OM intake (P < 0.05) and MP/CP intake increased (P < 0.05) with increasing feed intake.

A linear or quadric correlation was observed between MP supply (g/day) and the intake of OM (kg/day) or CP (g/day) (Table 4).

#### 4. Discussion

We observed a lower ratio of duodenal NAN/N intake in the AL group (0.87) compared with the 70% (1.04) and 50% (1.05) of AL groups. The lambs in the AL group may have had less recycled N in the form of urea than the lambs in the feed-restriction groups because the capacity for ammonia absorption was limited and excess ammonia could not be efficiently absorbed by the rumen. This may further explain the similarity in duodenal RUN flow between lambs in both the AL (10.2 g/day) and 70% (10.9 g/day)

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