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Full Length Research Paper

Effects of rumen-protected tryptophan on performance, nutrient utilization and plasma tryptophan in cashmere goats

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Thirty-six Liaoning cashmere goat wethers (28.72 ± 0.59 kg) were used to determine the effects of rumen-protected tryptophan (RPT) on performance, nutrient utilization and plasma tryptophan (Trp) during the cashmere fast-growing period. The goats were randomly assigned to the following treatments: Control (without RPT), LRPT (RPT – low), MRPT (RPT – medium) and HRPT (RPT – high) at RPT levels of 0, 2.0, 4.0 and 6.0 g per goat per day, respectively. RPT-medium supplementation improved average daily gain and feed efficiency ($P < 0.05$) when compared with the control and HRPT. RPT supplementation increased ($P < 0.05$) the length and growth rate of cashmere fiber, whereas no differences were observed among the LRPT, MRPT and HRPT treatment groups ($P > 0.05$). RPT-medium supplementation decreased urinary nitrogen (N) excretion and increased N retention when compared with other groups ($P < 0.05$). Plasma Trp concentration was higher for HRPT treatment group when compared with other treatments ($P < 0.05$). In conclusion, RPT supplementation potentially improved growth performance, N utilization and cashmere fibre growth in Liaoning cashmere goats. In the experimental conditions of the current trial, the optimum RPT supplementation level was 4.0 g per goat per day during the cashmere fast-growing period.

Key words: Rumen-protected tryptophan, growth performance, fibre characteristics, nutrient utilization, plasma tryptophan, cashmere goats.

INTRODUCTION

The essential amino acid tryptophan (Trp) is a required component of protein synthesis, it functions in the formation of serotonin and melatonin, and it can be a precursor for nicotinic acid. Deficiency of Trp induces depression of feed intake and growth performance in monogastric

animals (Montgomery et al., 1980; Henry et al., 1992). It is commonly assumed that most amino acids including Trp are not deficient in ruminants, because ruminal fermentation leads to production of microbial protein. The rumen microbial protein supply to the small intestines delivers a good quality pattern of essential and nonessential amino acids sufficient to support maintenance and normal, but not maximal, growth and milk production (Virtanen, 1966; Storm and Ørskov, 1984). It has been suggested that low supplementation or imbalanced ratio of Trp feeding could result to the deficiency of protein nutrition (Corfield and Robson, 1955; Reis and Tunks, 1978), which could lead to the insufficient supplement for maximum production (Virtanen, 1966; Storm and Ørskov, 1984). Under these conditions, supplemental feed sources high in rumen-protected Trp (RPT) can be fed to complement the supply of amino acids from microbial protein and balance the supply of essential amino acids

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Abbreviations: RPT, Rumen-protected tryptophan; Trp, tryptophan; LRPT, rumen-protected tryptophan – low; MRPT, rumen-protected tryptophan – medium; HRPT, rumen-protected tryptophan – high; DM, dry matter; BW, body weight; OM, organic matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADG, average daily gain.

#The first two authors contributed equally to this work.

to meet protein synthetic needs. Feeding on RPT has been shown to increase growth performance of lambs (Nolte et al., 2008) and the wool output of sheep (Reis and Colebrook, 1972), as well as improve nitrogen (N) utilization in goats (McLaren et al., 1965). However, the impacts of RPT on performance and nutrient utilization in cashmere goats are not yet known.

The LiaoNing Cashmere Goat is one of the major Cashmere goat in China which takes up 50% of the world's cashmere fiber yields (Zhang et al., 2008). Genetic factors as well as environmental effects had been widely investigated and discussed based on the cashmere fiber traits (Rose et al., 1992) whose seldom reports which focused on the nutritional treatments were provided in contrasts (Lvey et al., 2000). Studies have shown that Trp supplementation increases plasma melatonin levels in chickens, rats, ringdoves, heifers and humans (Herichova et al., 1998; Jaworek et al., 2003; Cubero et al., 2006; Kollmann et al., 2008; Hajak et al., 1991), and melatonin can stimulate cashmere fiber growth (Klören and Norton, 1995). Thus, RPT supplementation could be speculated to positively affect cashmere fibre growth. The objectives of this study were to investigate the effects of RPT on growth performance, fibre characteristics, nutrient utilization and plasma Trp in cashmere goats during the cashmere fiber fast-growing period.

MATERIALS AND METHODS

Animals, diets and feeding

The experiments were carried out at the Liaoning Cashmere Goats Breeding Center, China, from August to November 2008. The animal care, handling and sampling procedures were approved by the China Agriculture University Animal Care and Use Committee.

Thirty-six Liaoning Cashmere Goat wethers, averaging 1.0 years of age and 28.72 ± 0.59 kg initial body weight (BW), were stratified by weight and randomly assigned to the following treatments: Control (without rumen-protected tryptophan), LRPT (rumen-protected tryptophan – low), MRPT (rumen-protected tryptophan – medium) and HRPT (rumen-protected tryptophan – high) with 0, 2.0, 4.0 and 6.0 g RPT per goat per day, respectively. The RPT (containing 33% L-Trp, purchased from the Beijing Feeding Feed Science Technology Co., Beijing, China) was added to the concentrate diet. The levels of RPT supplementation were chosen based on the research of Itabashi et al. (1994). According to the conclusion of these researchers, supplementation at 4.0 g/day was suitable for research on RPT feeding. Based on the differences in weight and breed, we set two levels of 2.0 and 6.0 g/day supplementation, aiming to reveal the most feasible accession.

The experimental period lasted for 90 days, including a 10-day adaptation period and a 10-day metabolism trial. Goats were housed in an open-sided barn in individual pens (1.0 × 1.5 m). Fresh water was available at all time. Diets consisted of 60% Chinese wildrye hay, 10% alfalfa hay and 30% concentrate (on a dry matter (DM) basis) which was composed of minerals and vitamins (NRC, 2007) (Table 1). The concentrate mixture included corn and wheat bran as energy sources and soybean meal as a protein source. Hay was offered once a day *ad libitum*, and concentrate was fed 300 g daily and divided into two equal meals at 08:00 and 16:00 h.

Collection of data and samples

Daily feed offerings and refusals were measured to obtain feed intake for each goat. Body weight (BW) was obtained before the goats were fed in the morning on two consecutive days at the beginning and end of the experiment. During the last 10 days of the experiment, four goats from each treatment with similar feed intakes were housed individually in metabolism crates for the total collection of feces and urine. After a 5-day adapted period, collection of feces and urine was performed. Fecal was collected three times daily into fecal collection pans and stored at -20°C. At the end of each collection period, all feces from a goat was thawed, mixed thoroughly and subsampled for subsequent analysis. Urine was collected into plastic containers containing 50 ml of 50% HCl to prevent NH₃ volatilization. Urine volume was measured and recorded daily and a 10% aliquot was stored at -20°C until analysis.

Blood samples were collected on the day before RPT supplementation (day 0) and days 30, 60 and 90 via jugular venipuncture in heparinized-trace tubes before the goats were fed in the morning. Blood samples were immediately chilled to 0°C on ice and centrifuged at 4°C for 15 min at 3000 × g, then put in 5 ml polyethylene tubes and frozen at -20°C for analyses of plasma Trp concentration.

A 5 × 5 cm area of fiber on the right scapular area was shorn at the beginning of the study. On day 90, fiber samples were collected by stainless steel clippers from the 5 × 5 cm area. The samples were stored in sealed polythene bags at room temperature for cashmere fiber characteristics analyses.

Analytical procedures

All feed, orts and feces samples were dried at 55°C for 72 h in a forced air-drying oven, allowed to air-equilibrate, weighed to determine moisture loss and then ground in a Wiley mill to pass through a 1-mm screen (FZ102, Shanghai Hong Ji Instrument Co. Ltd, Shanghai, China). Dry matter, organic matter (OM) concentrations in feeds, orts and feces samples were analyzed using standard methods (AOAC, 1990). Nitrogen concentrations in feeds, feces and urine samples were determined by the Kjeldahl method (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured according to Van Soest et al. (1991). Tryptophan in feeds was analyzed according to the procedures described by Buraczewska and Buraczewski (1984). Plasma Trp concentration was measured using high-performance liquid chromatography system (LC-10A, HIMADZU, Japan) according to Pastuszewska (2007). Cashmere fibre samples were washed by soaking overnight in a detergent solution, rinsed thoroughly in deionized water and then dried at 80°C (Zhang et al., 2007). To calculate cashmere fibre growth, a calliper was used to measure the relaxed length (to the nearest millimetre) of the un-dyed section of the fibres at the end of experiment, and the diameter of cashmere fibre was measured on a random sample of 100 fibres using an Optic Fibre Diameter Analyse system (IWTO, 1992), as described by Shahabad et al. (1992).

Statistical analysis

The data were analyzed using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) (1988). The following model was used:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where, Y_{ij} is the dependent variables, μ is the overall mean, T_i is

Table 1. Ingredients and chemical composition of the basal diet.

| Item | Concentration |
|--|---------------|
| Ingredient (% , fed basis) | |
| Chinese wildrye hay | 60.0 |
| Alfalfa hay | 10.0 |
| Corn | 18.2 |
| Wheat bran | 3.00 |
| Soybean meal | 7.00 |
| Limestone | 0.20 |
| Dicalcium phosphate | 0.20 |
| NaCl | 1.00 |
| Premix ^a | 0.40 |
| Chemical composition (DM basis)^b | |
| Metabolizable energy (MJ/kg) | 10.1 |
| Crude protein (%) | 9.87 |
| Dry matter (%) | 91.7 |
| Neutral detergent fiber (%) | 50.5 |
| Acid detergent fiber (%) | 30.4 |
| Calcium (%) | 0.46 |
| Phosphorus (%) | 0.30 |
| Tryptophan (%) | 0.09 |

^aPremix contained the following per kilogram: FeSO₄·7H₂O, 170 g; CuSO₄·5H₂O, 70 g; MnSO₄·5H₂O, 290 g; ZnSO₄·7H₂O, 240 g; MgSO₄·H₂O, 120 g; Na₂SeO₃, 130 mg; KI, 220 mg; CoCl₂·6H₂O, 510 mg; vitamin A, 30,500,000 IU; vitamin D, 6,100,000 IU; vitamin E, 50,000 IU.
^bAnalyzed values except metabolizable energy. Metabolizable energy was calculated by metabolizable energy in ingredient of the basal diet.

the effect of RPT supplementation ($I = 1, 4$), ϵ_{ij} is the random error. Duncan's multiple range tests were used to detect statistical significance between treatment groups.

RESULTS

Performance

The effects of RPT supplementation on growth performance and cashmere fiber characteristics are shown in Table 2. There were no differences in initial and final BW among treatments ($P > 0.05$). Feed intake averaged 1183 g/day and was not affected ($P > 0.05$) by treatment. RPT–medium showed higher average daily gain (ADG) ($P < 0.05$) and lower feed efficiency ($P < 0.05$) but not significant with LRPT ($P > 0.05$). No differences among the control, LRPT and HRPT treatment groups ($P > 0.05$) were observed in ADG and feed efficiency.

There was no difference in cashmere fibre diameter between treatments ($P > 0.05$). However, cashmere growth length and cashmere growth rate were improved significantly ($P < 0.05$) by RPT supplementation but no differences among the LRPT, MRPT and HRPT treatment

groups ($P > 0.05$) were observed.

Nutrient utilization

The effects of RPT supplementation on digestibility of nutrients and N utilization are presented in Table 3. There were no differences in the digestibility of DM, OM, crude protein (CP), NDF and ADF among treatments ($P > 0.05$). Similarly, RPT supplementation had no influence on N intake and fecal N excretion among treatments ($P > 0.05$). However, MRPT supplementation decreased urinary N excretion ($P < 0.05$) and increased N retention ($P < 0.05$) when compared to other groups. LRPT also had less ($P < 0.05$) urinary N excretion and higher N ($P < 0.05$) retention than HRPT but was not significant with control, and no differences were found between control and HRPT group in urinary N excretion and N retention ($P > 0.05$).

Plasma tryptophan

Effects of RPT supplementation on plasma Trp concen-

Table 2. Effect of RPT supplementation on growth performance and fibre characteristics of cashmere goats.

| Item | Treatment ^a | | | | S.E.M |
|-------------------------------|------------------------|---------|--------|--------|-------|
| | Control | LRPT | MRPT | HRPT | |
| Initial live weight (kg) | 28.7 | 28.8 | 28.5 | 28.8 | 0.24 |
| Final live weight (kg) | 38.8 | 40.3 | 41.9 | 38.6 | 0.43 |
| Average daily gain (g) | 111.8b | 127.6ab | 148.9a | 108.9b | 8.62 |
| Feed intake (g/day) | 1126 | 1199 | 1215 | 1190 | 23.76 |
| Feed efficiency ^b | 10.08a | 9.38ab | 8.20b | 11.18a | 0.34 |
| Cashmere length (cm) | 3.68b | 4.71 a | 4.75a | 4.71a | 0.40 |
| Cashmere growth rate (mm/day) | 0.41b | 0.52a | 0.53a | 0.52a | 0.04 |
| Cashmere fiber diameter (µm) | 14.6 | 14.7 | 14.9 | 14.7 | 0.58 |

Means within the same row with different letters (a - b) are different ($P < 0.05$). ^aControl, LRPT, MRPT and HRPT at RPT levels of 0, 2.0, 4.0 and 6.0 g per goat per day, respectively. ^bFeed efficiency was calculated by the formula: Feed intake (g/day) / average daily gain (g); S.E.M, standard error of mean.

Table 3. Effect of RPT supplementation on digestibility of nutrients and N utilization of cashmere goats.

| Item | Treatment ^a | | | | S.E.M |
|------------------------------|------------------------|-------|-------|-------|-------|
| | Control | LRPT | MRPT | HRPT | |
| Digestibility (%) | | | | | |
| DM | 70.0 | 71.4 | 75.1 | 68.8 | 1.95 |
| OM | 71.2 | 72.1 | 75.8 | 69.1 | 2.21 |
| CP | 71.6 | 74.3 | 76.5 | 70.3 | 1.69 |
| NDF | 64.3 | 65.7 | 66.2 | 64.8 | 0.78 |
| ADF | 60.1 | 62.0 | 62.6 | 60.0 | 1.00 |
| N utilization (g/day) | | | | | |
| N intake | 15.9 | 16.8 | 17.0 | 16.8 | 0.29 |
| Fecal N | 4.67 | 4.50 | 3.99 | 4.91 | 0.30 |
| Urinary N | 5.23ab | 5.04b | 4.01c | 5.92a | 0.21 |
| N retention | 5.98cb | 7.27b | 9.01a | 5.94c | 0.48 |

Means within the same row with different letters (a - c) are different ($P < 0.05$). ^aControl, LRPT, MRPT and HRPT at RPT levels of 0, 2.0, 4.0 and 6.0 g per goat per day, respectively; S.E.M, standard error of mean.

Table 4. Effect of RPT supplementation on plasma Trp concentrations of cashmere goats (µg/ml).

| Day | Treatment ^a | | | | S.E.M |
|-----|------------------------|---------|---------|---------|-------|
| | Control | LRPT | MRPT | HRPT | |
| 0 | 12.02 | 13.48 | 12.92 | 13.12 | 0.11 |
| 30 | 12.61 b | 15.06 b | 16.65 b | 21.49 a | 0.23 |
| 60 | 12.91 b | 15.38 b | 16.40 b | 21.18 a | 0.15 |
| 90 | 12.31 b | 16.03 b | 17.05 b | 22.12 a | 0.06 |

Means within the same row with different letters (a - b) are different ($P < 0.05$). ^aControl, LRPT, MRPT and HRPT at RPT levels of 0, 2.0, 4.0 and 6.0 g per goat per day, respectively; S.E.M, standard error of mean.

tration are shown in Table 4. At the beginning of the experiment, there was no difference in plasma Trp concentration among treatments ($P > 0.05$). However, plasma Trp concentrations on day 30, 60 and 90 of the experiment were increased by RPT supplementation and were higher ($P < 0.05$) for HRPT group when compared with other groups, and no differences among the control, LRPT and MRPT treatment groups ($P > 0.05$)

were observed..

DISCUSSION

Performance

The ADG increased with MRPT supplementation, and

then declined at the high level of RPT supplementation. These effects were due to the metabolic balance of amino acid *in vivo*, which resulted in a high level of protein synthesis. Nolte et al. (2008) found Trp to be the third most limiting amino acid in growing lambs during the process of non-protein N utilization. They also found that supplementation of Trp in a rumen-protected form had positive effects on growth performance in ruminants. However, higher Trp supplementation might have had a depressive effect on growth by causing an amino acid imbalance or an amino acid antagonism (Candlish et al., 1970). The present results indicated that 4.0 g/d RPT supplementation in a cashmere goat diet was adequate to meet the optimal dietary requirements; growth performance was not further improved by a higher supply of RPT.

Typical cashmere fiber diameter in Mongolia, China and Iran ranges between 13 and 16 μm (Millar, 1986). Cashmere fiber diameters observed in this experiment are within the range of 13 - 16 μm and RPT supplementation improved cashmere fiber growth. Reis and Colebrook (1972) found that the addition of L-Trp to the zein could increase wool yield in sheep. The amount of amino acids reaching the intestine may affect cashmere fiber growth (Hynd and Allden, 1985). Besides the effect of the absorbed amino acid on the cashmere fiber growth, higher Trp availability could have exerted an effect also on the endocrine system involved on cashmere fiber growth. In fact, it has been observed that free plasma Trp penetrates through the blood-brain barrier into the brain, within pineal cells, it is converted to melatonin (Kollmann et al., 2008). Studies have also shown that Trp supplementation can increase plasma melatonin levels (Herichova et al., 1998; Jaworek et al., 2003; Cubero et al., 2006; Kollmann et al., 2008; Hajak et al., 1991), and melatonin can stimulate cashmere fiber growth (Klören and Norton, 1995). Based on the length and growth rate of cashmere fiber, RPT supplementation may be an effective means of improving the cashmere fiber growth.

Nutrient utilization

The result demonstrated that the RPT supplementation improved N utilization by increasing N retention. Similar observations have been obtained by Itabashi et al. (1994) that N retention was increased by 4 g/d RPT supplementation to Japanese Saanen Goats. An increased retention of absorbed N was observed by McLaren et al. (1965) when 0.8 g L-Trp was substituted for an isonitrogenous amount of urea in a lamb ration. Nolte et al. (2008) found that N retention of lambs was limited by inadequate supplies of absorbable Trp, and supplementation of Trp in a rumen-protected form could be beneficial to growing lambs fed diets low in ruminally undegradable protein. An increase in N retention was due to less urinary N excretion. Nimrick et al. (1970) reported

that all responses in N retention of lambs abomasally administered amino acids resulted from changes in urinary N excretion. In this experiment, the decreased urinary N excretion and the increased N retention when 4.0 g/day RPT was supplemented indicated that RPT supplementation may improve amino acid balance, resulting in greater protein synthesis and decreased N excretion.

Plasma Trp

Studies have shown that Trp supplementation results in an increase of plasma Trp concentrations under certain experimental conditions. Kollmann et al. (2008) found that 125 g/day Trp supplementation caused an increase of plasma Trp levels in heifers and in cows both at day and night time. Koopmans et al. (2006) indicated that pigs fed Trp supplemented diet had approximately a 2-fold elevation in plasma Trp concentrations on days 4, 5 and 6 after diet introduction. When provision of an essential amino acid is below the requirement, the amino acid concentration in plasma may either increase marginally or not at all. However, once the requirement for the essential amino acid has been met, plasma concentration will increase more rapidly (Bergen, 1979; Broderick et al., 1974). In the present study, the plasma Trp increased only slowly at 4.0 g/day RPT supplementation, but increased rapidly at 6.0g/day RPT supplementation. This indicated that the Trp requirements of the cashmere goats used in the present study were being adequately met by 4.0 g/day RPT supplementation.

Conclusion

It can be concluded that that RPT supplementation improved the ADG and N utilization of cashmere goats, and may be an effective means of improving the cashmere fiber growth. Based on the present findings, the beneficial level of long-term RPT supplementation for Liaoning cashmere goat wethers is 4.0 g/day during the cashmere fast-growing period.

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