ruminants

MDPI

Article

Supplementing Forage with Traditional Chinese Medicine Can Increase Microbial Protein Synthesis in Sheep

Xi Liang [†], **Xue Bi**, **Hiroaki Sano** and **Jin Jin** [*,†]

Department of Animal Science, Iwate University, Morioka 020-8550, Japan
* Correspondence: msj.jin@hotmail.com
† Current address: Shanwei Academy of Agricultural Sciences, Shanwei 516600, China.

Simple Summary: Antibiotic growth promoters are encountering diminishing social acceptance due to escalating public concern regarding the potential risks of drug residues to human health. Traditional Chinese medicine (TCM), with its extensive history of use for human health, has been validated as safe and effective through numerous clinical practices. TCM encompasses a wide range of natural herbs and plants that are used to treat or prevent diseases or promote health. The natural constituents of TCM minimize the risk of drug residue accumulation, making it a potential alternative to antibiotics in livestock feed. The present study introduced a TCM extract as a potential substitute for antibiotics that could improve feed efficiency and alter nutrient metabolism in sheep. The findings offer significant data for advancing research into TCM's application in boosting ruminant productivity.

Abstract: Traditional Chinese medicine (TCM) encompasses a wide range of natural herbs and plants that are used to treat or prevent diseases or promote health. This study aimed to evaluate the effect of feeding a TCM formula extract on nitrogen (N) balance, microbial nitrogen supply (MNS), and plasma leucine kinetics in sheep. Six sheep were fed with mixed hay (Hay-diet) only or supplemented with 2% TCM (mixture of Astragalus root, Angelica root, and Atractylodes rhizome; TCM-diet) in a crossover design over two 21-day periods. An isotope dilution of [1-^{13}C]leucine was used to measure the rate of plasma leucine turnover. Purine derivative (PD) excretion in urine was determined to estimate the MNS. The TCM-diet significantly increased N intake (p < 0.01) and N digestibility (p = 0.02) compared to the Hay-diet, with less N excretion (p = 0.02) in feces, while no significant difference was observed between diets in terms of N excretion in urine and N retention. Total PD excretion and MNS were higher (p < 0.01), as well as the turnover rate of plasma leucine tended to be higher (p = 0.06) with the supplementation of TCM. The present results suggested that the TCM formula could be considered as a potential feed additive for ruminant production.

Keywords: [1-^{13}C]leucine; leucine turnover; microbial protein; nitrogen balance; sheep; traditional Chinese medicine

Citation: Liang, X.; Bi, X.; Sano, H.; Jin, J. Supplementing Forage with Traditional Chinese Medicine Can Increase Microbial Protein Synthesis in Sheep. *Ruminants* **2024**, *4*, 613–622. https://doi.org/10.3390/ruminants4040042

Academic Editor: Uchenna Y. Anele

Received: 16 November 2024
Revised: 14 December 2024
Accepted: 17 December 2024
Published: 19 December 2024

1. Introduction

In ruminants, microorganisms synthesize microbial protein from dietary protein and non-protein nitrogen (N) compounds in the rumen. Approximately 50% of the protein was utilized by the animals sourced from microbial protein synthesis [1], which also provides 50% to 80% of the total absorbable protein supplied to the small intestine [2]. Microbial protein is highly digestible in the small intestine and contributes about two-thirds of the amino acids absorbed by ruminants [3]. With the expansion of animal production, ensuring an adequate supply of amino acids from microbial protein holds considerable significance for sustaining enhanced growth, lactation, and wool production. Consequently, optimizing microbial protein synthesis in the rumen becomes a critical factor.

The application of antibiotic feed additives to enhance the productivity of ruminants is a common practice worldwide, with the aim of achieving better growth performance or milk production. However, their use is facing reduced social acceptance due to the presence of antibiotic residues in animal products, which is a risk to human health. Traditional Chinese medicine (TCM) has received increasing attention as a promising alternative source to antibiotics for animal production during the past two decades [4]. Meanwhile, recent studies have shown that some TCM additives containing bioactive compounds such as flavonoids, polyphenols, and polysaccharides can modulate immune responses and intestinal health in pigs [5,6] while improving growth and nutrient digestibility in ruminants [7,8], demonstrating great potential as substitutes for dietary antibiotics. The livestock industry is in pressing need of effective alternatives to antibiotics, and TCM offers a promising avenue for the development of novel feed additives aimed at mitigating and reducing drug residues in animal-derived food products. TCM additives, commonly defined as Chinese herbs or their crude extracts (aqueous dissolved phytogenic compounds), are utilized as dietary supplements at subclinical doses in healthy animals for both nutritional and healthcare purposes, exhibiting minimal to no side effects [9]. The TCM comprises numerous plant species with various functions and is often utilized in formulae to obtain synergistic effects or to diminish possible adverse reactions [10]. Astragalus root (*Astragalus membranaceus*), Angelica root (*Angelica sinensis*), and Atractylodes rhizome (*Atractylodes lancea*) are three abundant and popular Chinese herbs being extensively used in medicinal treatment. The herbs are known to contain a variety of bioactive components such as polysaccharides (Astragalus root) and essential oils (Angelica root and Atractylodes rhizome), which feature neuroprotective, anti-inflammatory, and immune stimulatory properties [11–13]. The combination of the three herbs makes a formula that serves as a supplement for humans to relieve stress and tiredness [14].

A couple of research studies were to be formulated using Astragalus root, Angelica root, and Atractylodes rhizome on different nutritional parameters to introduce a potential feed for ruminant production. In the previous experiment conducted by the present research group, the supplementation of this TCM formula extract to a hay diet was found to improve ruminal fermentation of dietary carbohydrates and enhance whole-body protein metabolism in sheep [15]. The TCM formula was assumed to improve the efficiency of microbial protein synthesis and promote intestinal amino acid absorption. To this end, the present study was conducted to assess the effect of feeding TCM on N balance, microbial N supply (MNS), and rate of plasma leucine turnover in sheep.

2. Materials and Methods

2.1. Preparation of TCM

The TCM used in this experiment was purchased from a licensed drug market (China) and involved Astragalus root (55%), Angelica root (27%), and Atractylodes rhizome (18%). The herbal proportions were aligned with the traditional Chinese pharmacopeia. The raw materials were air-dried, chopped, and subjected to boiling for 20 min, a process repeated three times and pooled to yield the liquid extract.

2.2. Animals, Diets, and Management

The experiment was conducted at the Faculty of Agriculture, Iwate University, in Morioka (39°42′ N, 141°09′ E). Six healthy crossbred (Corriedale × Suffolk) adult shorn wethers with a mean body weight (BW) of 51 ± 3 kg were selected for subsequent use at the start of the experiment. The sheep were randomly divided into two experimental groups, and two dietary treatments were tested. One group received mixed hay (Hay-diet (dry matter 86.6%, metabolizable energy 1760 kcal/kg, and crude protein (CP) 116 g/kg) of orchard grass (*Dactylis glomerata*) and reed canary grass (*Phalaris arundinacea*) at maintenance level [16]), and another group received the Hay-diet supplemented with 2% (dry matter weight of hay) TCM (TCM-diet; CP 24 g/L). The mixed hay was administered at 67 g/kg $BW^{0.75}$ per day in both dietary treatments. The TCM dosage was determined based on the ratio of crude herbs to the volume of boiling water (approximately 100 g to 325 mL; 85 mL/head per day). The experiment was performed using a crossover design over two 21-day periods. One sheep group was fed the Hay-diet during the first period, followed by the TCM-diet, while the other group was fed in the reverse order. During the pre-feeding period (the first 14 days), the animals were housed individually in pens within an animal barn. Subsequently, they were transferred to metabolic cages in a temperature-controlled environment at 23 ± 1 °C, with a lighting regimen from 08:00 to 22:00 h. Diets were given twice a day at 08:30 h and 20:30 h, and clean, fresh water was available ad libitum. The TCM was injected into animal's mouth by a syringe before giving the mixed hay. The sheep were weighed on the day of starting experiment and every 7 days of each dietary period.

2.3. Collection of Urine, Feces, and Rumen Fluid

N balance trial was carried out from day 18 to 20 of each 21-day treatment period. Urine and feces samples were taken from each sheep at 24 h intervals. Urine was collected with a plastic bucket containing 50 mL of 6 *N* H_2SO_4 solution in order to prevent N loss, the total volume was recorded, and the pH value of urine was checked (below 3) with a pH meter (F-51, HORIBA, Kyoto, Japan); then, subsamples (50 mL) were taken for further processing. To determine purine derivatives (PD) excretion, 5 mL of urine was taken from each subsample and diluted with 20 mL of water to prevent precipitation. The diluted urine samples and remaining subsamples were stored at −30 °C for later analysis. The collected feces were dried in a forced-air oven at 60 °C for 48 h and then placed at room temperature for 5 days. The air-dried samples were weighed, and subsamples were ground for later analysis.

Rumen fluid was collected on day 20 of each treatment period. Sample (30 mL) was taken three times from each sheep with an orally inserted stomach tube at 0 h (before feeding), 3 h, and 6 h after feeding. A portion of sample was centrifuged at 8000× *g* for 10 min at 4 °C (RS-18IV, Tomy, Tokyo, Japan), and then 1 mL of supernatant was taken and mixed with 1 mL of 0.1 *N* HCl to determine ammonia concentration. The prepared samples were stored at −30 °C until chemical analysis.

2.4. Isotope Dilution Method

An isotope dilution technique using [1-^{13}C]leucine was conducted to determine plasma leucine kinetics in sheep on day 21 of each treatment period. For isotope infusion and blood collection, two polyvinyl catheters filled with sterile solution of 3.8% trisodium citrate were inserted into both jugular veins at least two hours prior to starting experiment. At 12:00 h, a saline solution (0.9% sodium chloride) containing 7.2 µmol/kg $BW^{0.75}$ of [1-^{13}C]leucine (L-leucine-1-^{13}C, 99 atom% excess ^{13}C; Cambridge Isotope Laboratories, USA) was injected into the infusion catheter as a priming dose. Immediately after the injection of priming dose, [1-^{13}C]leucine was continuously infused at rate of 7.2 µmol/kg $BW^{0.75}$/h by a multichannel peristaltic pump (AC-2120, Atto, Tokyo, Japan) for 4 h. Blood samples were taken through the sampling catheter before priming injection (12 mL) and at 30 min intervals (6 mL) over the last 120 min of [1-^{13}C]leucine infusion. The collected samples were transferred to heparinized tubes and temporarily kept in crushed ice until centrifugation. After the end of the experiment, blood samples were centrifuged at 10,000× *g* for 10 min at 4 °C, and the plasma was stored at −30 °C for later analysis.

2.5. Chemical Analysis

N contents in diets, urine, and feces were analyzed by the Kjeldahl method with a Foss Kjeltec System (Tecator 2520 and Kjeltec 2300, Foss, Hoganas, Sweden). Ruminal ammonia concentration was measured using a colorimetric method [17]. The PD excretion, including allantoin, uric acid, and xanthine plus hypoxanthine from diluted urine samples, was determined by spectrophotometer (V-630 BIO, JASCO, Tokyo, Japan) according to the procedure of Chen and Gomes [18]. Plasma free amino acids were determined with an automatic amino acid analyzer (JLC-500/V, JEOL, Tokyo, Japan) based on high-performance liquid chromatography. Amino acids and α-keto acids were separated and converted to the *t*-butyldimethylsilyl derivatives; then, plasma α-ketoisocaproic acid (α-KIC) concentration and plasma α-[1-^{13}C]KIC enrichment were measured using a gas chromatography–mass spectrometry (QP-2010, Shimadzu, Kyoto, Japan) with selected ion monitoring, as reported by Sano et al. [19].

2.6. Calculations

The PD excretion and the MNS were calculated using the equations given by Chen and Gomes [18].

The amount of microbial purines absorbed (X; mmol/d) corresponding to the PD excreted (Y; mmol/d) was calculated as follows:

$$Y = 0.84\ X + (0.150BW^{0.75}\ e^{-0.25X})$$

where 0.84 is the proportion of absorbed exogenous purine excreted as derivatives in the urine, and the component within parenthesis represents the endogenous derivative excretion. The calculation of X from Y based on the equation was performed by means of the Newton–Raphson iteration process.

The MNS (g/d) was calculated from the following equation:

$$MNS = X \times 70/0.116 \times 0.83 \times 1000$$

where 70 is the N content of purines (mg/mmol), 0.116 is the ratio of purine N to total N in mixed rumen microbes, and 0.83 is the digestibility of microbial purines.

The turnover rate of plasma leucine (LeuTR; μmol/kg $BW^{0.75}$/h) was calculated according to the equation described by Wolfe [20]:

$$LeuTR = I \times (1/E - 1)$$

where I represents the infusion rate of [1-^{13}C]leucine, and E represents the plasma isotopic enrichment of α-[1-^{13}C]KIC at steady state.

2.7. Statistical Analysis

All data were statistically analyzed using analysis of variance with the MIXED procedure of SAS (SAS software, version 6.11; SAS Institute Inc., Cary, NC, USA). The fixed effects in the model were period and diet, and the random effect was sheep. Only diet effect was considered for the results, as no significant period effect was detected in the parameters. Results were defined as significant at the $p < 0.05$ level, and a tendency was at $0.05 \leq p < 0.10$.

3. Results

3.1. Body Weight Gain, N Balance, and Ruminal Ammonia Concentration

The sheep consumed all the diets that were fed. Body weight gain did not differ between dietary treatments (Table 1). N intake was higher ($p < 0.01$) for the TCM-diet than for the Hay-diet. N excretion in feces was lower ($p = 0.02$) for the TCM-diet than the Hay-diet, but N excretion in urine and N retention did not differ between diets. N digestibility was higher ($p = 0.02$) for the TCM-diet compared to the Hay-diet. The concentration of ruminal ammonia tended to be higher ($p = 0.08$) for the TCM-diet than for the Hay-diet.

Table 1. Effect of traditional Chinese medicine on body weight gain, nitrogen (N) balance, and ruminal ammonia concentration in sheep.

	Hay-Diet	TCM-Diet	SEM	*p*-Value
No. of sheep	6	6	-	-
Body weight gain (g/d)	21	29	9.2	0.19
Parameters of N balance (g/kg BW$^{0.75}$/d)				
N intake	1.27	1.29	0.007	<0.01
N in feces	0.50	0.46	0.033	0.02
N in urine	0.46	0.47	0.068	0.65
N retention	0.31	0.35	0.042	0.17
N digestibility (%)	61	64	1.1	0.02
Ruminal ammonia (mmol/L)	10.0	11.9	0.92	0.08

Hay-diet, mixed hay of orchard grass and reed canary grass; TCM-diet, Hay-diet supplemented with 2% traditional Chinese medicine; SEM, standard error of the mean.

3.2. Plasma Free Amino Acids

Plasma amino acids determined in the pre-infusion period of the isotope dilution method are shown in Table 2. Concentrations of plasma threonine and glycine were lower ($p < 0.05$), and those of isoleucine, leucine, and serine tended to be lower ($p < 0.10$) for the TCM-diet than the Hay-diet.

Table 2. Effect of traditional Chinese medicine on concentrations of plasma free amino acids at pre-infusion period in sheep.

	Hay-Diet	TCM-Diet	SEM	*p*-Value
No. of sheep	6	6	-	-
Plasma amino acids (μmol/L)				
Threonine	245	210	16.8	0.03
Valine	297	285	13.6	0.14
Methionine	29	30	6.4	0.30
Iso-leucine	116	83	10.0	0.06
Leucine	160	111	19.1	0.08
Phenylalanine	60	51	4.1	0.34
Histidine	68	79	14.7	0.27
Lysine	95	74	10.7	0.12
Serine	178	139	25.3	0.06
Asparagine	50	47	5.0	0.41
Glutamic acid	91	88	10.8	0.45
Glutamine	278	259	29.1	0.19
Glycine	492	422	33.3	0.04
Alanine	212	189	17.7	0.17
Tyrosine	74	80	3.4	0.51
Tryptophan	44	41	2.0	0.60
Arginine	160	143	13.2	0.17
Proline	101	84	20.3	0.33

Hay-diet, mixed hay of orchard grass and reed canary grass; TCM-diet, Hay-diet supplemented with 2% traditional Chinese medicine; SEM, standard error of the mean.

3.3. PD Excretion, MNS, and Plasma Leucine Kinetics

Urinary allantoin, uric acid, xanthine plus hypoxanthine, and total PD excretion in the urine were higher ($p < 0.01$) for the TCM-diet than for the Hay-diet. The MNS was also greater ($p < 0.01$) for the TCM-diet compared to the Hay-diet (Table 3). Concentration of plasma α-KIC and enrichment of plasma α-[1-^{13}C]KIC were stable during the latter period (2 h) of [1-^{13}C]leucine infusion (Figure 1). The concentration of plasma α-KIC tended to be lower ($p = 0.09$) for the TCM-diet than for the Hay-diet. Plasma LeuTR tended to be higher ($p = 0.06$) for the TCM-diet compared to the Hay-diet (Table 3).

Table 3. Effect of traditional Chinese medicine on purine derivatives (PD) excretion, microbial nitrogen supply (MNS), and plasma leucine kinetics in sheep.

	Hay-Diet	TCM-Diet	SEM	*p*-Value
No. of sheep	6	6	-	-
Parameters of PD excretion (mmol/kg BW$^{0.75}$/d)				
Allantoin	0.34	0.46	0.019	<0.01
Uric acid	0.03	0.05	0.006	<0.01
Xanthine + hypoxanthine	0.05	0.06	0.006	<0.01
Total PD	0.43	0.57	0.023	<0.01
MNS (g/kg BW$^{0.75}$/d)	0.36	0.49	0.021	<0.01
Plasma leucine kinetics				
α-KIC concentration (μmol/L)	14.7	12.9	1.17	0.09
LeuTR (μmol/kg BW$^{0.75}$/h)	373	437	16.3	0.06

Hay-diet, mixed hay of orchard grass and reed canary grass; TCM-diet, Hay-diet supplemented with 2% traditional Chinese medicine; SEM, standard error of the mean; α-KIC, α-ketoisocaproic acid; LeuTR, turnover rate of plasma leucine.

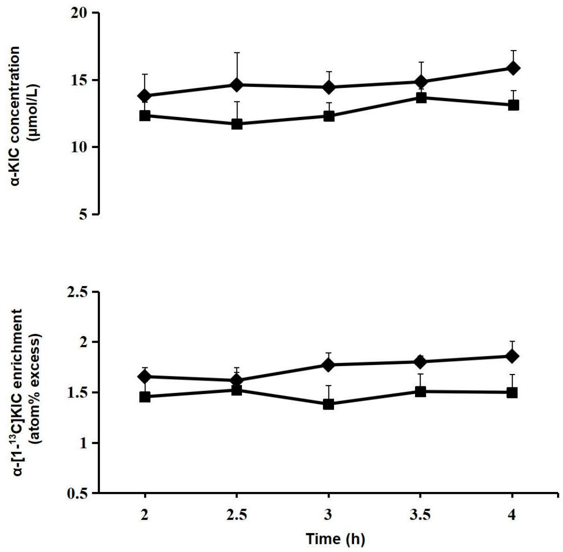

Figure 1. Time course of changes in plasma α-ketoisocaproic acid (α-KIC) concentration and plasma α-[1-^{13}C]KIC enrichment during the last 2 h of [1-^{13}C]leucine infusion in sheep fed two different diets (♦ = Hay-diet; ■ = TCM-diet).

4. Discussion

The TCM-diet increased both N intake and N digestibility compared to the Hay-diet, involving less N excretion in feces. The N intake in the TCM-diet was 0.02 g/kg BW$^{0.75}$/d higher due to the extra N supplied by the extract. Previous studies [19,21] on N balance in ruminants revealed the significant impact of the dietary intake of CP on N digestibility. The differences in CP intake in this experiment were minimal, suggesting that they were unlikely to significantly affect N digestibility. Therefore, the observed increase in N digestibility might be attributed to the bioactive components present in the TCM-diet. Essential oils, a major bioactive component of Angelica root and Atractylodes rhizome, are recognized for their antimicrobial properties against a broad range of microorganisms, including Gram-positive and Gram-negative bacteria, fungi, and viruses [22]. Essential oils have been well studied for their potentiality as modifiers of rumen microbial fermentation [23] and are considered a useful means to improve the efficiency of nutrient utilization in ruminants [24]. Specifically, Soltan et al. [25] reported that the apparent digestibility of dietary protein was slightly improved with the supplementation of essential oils in dairy cows. A previous experiment in cattle by Yang et al. [26] showed that compared to the control, feeding essential oils could increase ruminal digestion of dietary protein by 11%. Hart et al. [27] discussed the positive effects of essential oils on ruminal fermentation and stated that the main effect of essential oils in rumen was to manipulate protein and amino acid degradation through selective action on certain rumen microorganisms. Therefore, TCM components, including essential oils, were posited to significantly influence ruminal fermentation and enhance N digestibility in sheep. Moreover, the metabolizable energy was considered to be comparable between the two experimental diets due to the limited TCM amount. Fujita et al. [28] reported that N retention would be more related to metabolizable energy intake compared to the N intake, which might justify the absent response in N retention in the current study.

Ruminal ammonia N stands out as the most important N source for microbial protein synthesis in the rumen [29]. In the present experiment, ruminal ammonia concentration tended to be higher for the TCM-diet compared to the Hay-diet, indicating that higher quantities of N could be available for microbial protein synthesis in sheep fed with the TCM-diet. The urinary excretion of PD, an important index of microbial protein status in ruminants, was employed to estimate the MNS [30]. In previous studies, it was found that the efficiency of MNS changed with dietary intake [31], and the MNS increased with enhanced N digestibility in sheep [32]. These findings were in good agreement with the present observation. Presently, the effect of TCM on microbial protein synthesis has been rarely reported in both in vivo and in vitro studies. As reported previously [26,33,34], essential oils had no effect on ruminal ammonia concentration and microbial protein synthesis. Astragalus polysaccharide serves as a bioactive component extracted from the stem or root of *Astragalus membranaceus*. Zhong et al. [35] examined the effect of feeding Astragalus polysaccharide on rumen fermentation in lambs and found that Astragalus polysaccharide significantly increased ruminal ammonia concentration. Yin et al. [36] reported that Astragalus polysaccharide could ameliorate the digestive function and improve the digestion of dietary protein in piglets. Accordingly, it was also suggested that Astragalus polysaccharide might enhance the ruminal proteolysis of dietary protein and subsequently supply higher quantities of N to rumen microorganisms, resulting in an increase in microbial protein synthesis. Moreover, the TCM was found to enhance whole-body protein synthesis in sheep in a previous study conducted by the present research group [15]. Hence, the result from the current experiment demonstrated that the bioactive components of TCM, such as Astragalus polysaccharide and essential oils, might have a positive effect on ruminal N metabolism, thereby enhancing microbial protein supply and intestinal protein digestion.

In the current experiment, concentrations of numerous plasma free amino acids were reduced or tended to be lower in the TCM-diet compared to the Hay-diet. Various factors are responsible for affecting the level of free amino acids in plasma, such as microbial protein synthesis and amino acid absorption [37]. Increased microbial protein supply would result in increased absorption of amino acid into enterocytes because microbial protein is highly digestible in the small intestine [2]. Generally, a reduction in plasma amino acid concentration may indicate an increased utilization of amino acids for protein synthesis in ruminants [38]. In a previous experiment by the present research group [15], a decrease in plasma amino acid concentration was observed in response to enhanced whole-body protein synthesis in sheep with the same diets applied. Therefore, the lower concentrations of numerous plasma amino acids in the TCM-diet might be attributed to enhanced intestinal absorption of these amino acids, coupled with a greater incorporation of such amino acids into whole-body protein synthesis in sheep.

In the present study, the enrichment of plasma α-[1-^{13}C]KIC was used to calculate LeuTR instead of plasma [1-^{13}C] leucine due to its role as the true precursor of intracellular leucine metabolism [39]. The trend of increased LeuTR for the TCM-diet was comparable to the previous findings [15], where the TCM supplementation improved the rates of plasma phenylalanine and tyrosine turnover in sheep. Actually, the health-beneficial activity of TCM on the human body is to maintain and restore energy balance by inducing hematopoiesis. The action of TCM on the blood system is acknowledged to accelerate the generation, growth, and maturity of blood cells [40]. Meanwhile, the distribution, absorption, and metabolism of TCM in the body must be combined with plasma protein [41]. Thus, the changes in plasma amino acid kinetics in sheep might be largely attributed to the influence of protein binding to TCM components in plasma and the impact of these bioactive components on hematopoiesis. Yin et al. [36] stated that Astragalus polysaccharide could regulate amino acid metabolism by accelerating the entry of dietary amino acid into the systemic circulation in piglets. A previous study in humans by Li et al. [42] demonstrated that the combination of Astragalus root and Angelica root enhanced protein turnover in nephrotic patients. Their research findings agree with the current observations in sheep. Furthermore, microbial protein supply to the lower gut contributes about two-thirds of the amino acids absorbed by ruminants [3], and the increased LeuTR might also result from a higher supply of microbial protein as the source of absorbable amino acids from the small intestine to the tissues to support amino acid turnover. As reported by Lapierre et al. [43], increasing metabolizable protein (the microbial protein or undegraded dietary protein that can be absorbed by the intestine) supply in lactating cows leads to an increase in net leucine and LeuTR absorption. Fujita et al. [28] reported that by enhancing microbial protein synthesis in the rumen and amino acid absorption, the supplementation of starch increased plasma phenylalanine and tyrosine turnover rates in adult goats, further justifying the observation of the present study.

5. Conclusions

The present findings suggested that the TCM formula could serve as a feed additive to enhance microbial protein supply and improve N utilization in sheep. To capture the full potential of the TCM formula for ruminant production, further experiments are still warranted to delve into its performance in growing, pregnant, or lactating ruminants.

Author Contributions: Conceptualization, X.L. and J.J.; methodology, X.L. and H.S.; formal analysis, X.L. and X.B.; investigation, X.B.; resources, H.S.; data curation, X.L.; writing—original draft preparation, X.L.; writing—review and editing, H.S. and J.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The handling of experimental animals, including cannulation and blood collection, was reviewed and approved by Iwate University (Approval no. A201310). All experimental procedures were carried out according to the guidelines established by the Animal Care Committee of Iwate University.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated or analyzed during this study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors wish to thank Najiaowa Yu (China Scholarship Council Ph.D. scholarship), University of Alberta, Canada, for her kind comments on the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Harmon, D.L.; Swanson, K.C. Review: Nutritional regulation of intestinal starch and protein assimilation in ruminants. *Animal* **2020**, *14*, s17–s28. [CrossRef] [PubMed]
2. Stern, M.D.; Bach, A.; Calsamiglia, S. New concepts in protein nutrition of ruminants. In Proceedings of the 21st Annual Southwest Nutrition and Management Conference, Tempe, AZ, USA, 23–24 February 2006; pp. 45–66.
3. Pathak, A.K. Various factors affecting microbial protein synthesis in the rumen. *Vet. World* **2008**, *1*, 186–189.
4. Abdallah, A.; Zhang, P.; Zhong, Q.Z.; Sun, Z.W. Application of traditional Chinese herbal medicine by-products as dietary feed supplements and antibiotic replacements in animal production. *Curr. Drug Metab.* **2019**, *20*, 54–64. [CrossRef] [PubMed]
5. Lien, T.F.; Horng, Y.M.; Wu, C.P. Feasibility of replacing antibiotic feed promoters with the Chinese traditional herbal medicine Bazhen in weaned piglets. *Livest. Sci.* **2007**, *107*, 97–102. [CrossRef]

6. Yin, F.; Yin, Y.; Kong, X.; Liu, Y.; He, Q.; Li, T.; Huang, R.; Hou, Y.; Shu, X.; Tan, L.; et al. Dietary supplementation with *Acanthopanax senticosus* extract modulates gut microflora in weaned piglets. *Asian-Aust. J. Anim. Sci.* **2008**, *21*, 1330–1338. [CrossRef]
7. Lien, T.F.; Liao, C.M.; Lin, K.J. Effects of supplemental Chinese traditional herbal medicine complex on the growth performance, carcass characteristics, and meat quality of male *Holstein calves*. *J. Appl. Anim. Res.* **2014**, *42*, 222–227. [CrossRef]
8. Liu, X.; Liu, F.; Yan, T.; Chang, S.; Wanapat, M.; Hou, F. *Cistanche deserticola* addition improves growth, digestibility, and metabolism of sheep fed on fresh forage from alfalfa/tall fescue pasture. *Animals* **2020**, *10*, 668. [CrossRef]
9. Gong, J.; Yin, F.; Hou, Y.; Yin, Y. Review: Chinese herbs as alternatives to antibiotics in feed for swine and poultry production: Potential and challenges in application. *Can. J. Anim. Sci.* **2014**, *94*, 223–241. [CrossRef]
10. Jin, Y.; Qu, C.; Tang, Y.; Pang, H.; Liu, L.; Zhu, Z.; Shang, E.; Huang, S.; Sun, D.; Duan, J.A. Herb pairs containing *Angelicae Sinensis* Radix (Danggui): A review of bio-active constituents and compatibility effects. *J. Ethnopharmacol.* **2016**, *181*, 158–171. [CrossRef]
11. Chen, L.L.; Wang, Y.H.; Qi, J.; Dong, T.X.; Zhu, D.N.; Yu, B.Y. Identification and determination of absorbed components of Danggui-Shaoyao-San in rat plasma. *Chin. J. Nat. Med.* **2011**, *9*, 363–368. [CrossRef]
12. Zhou, J.; Fang, L.; Wang, X.; Zhang, J.; Guo, L.P.; Huang, L.Q. Comparison of the volatile compounds of crude and processed *Atractylodis rhizome* analyzed by GC-MS. *Afr. J. Pharm. Pharmacol.* **2012**, *6*, 2155–2160. [CrossRef]
13. Gopi, S.; Amalraj, A.; Kunnumakkara, A.; Thomas, S. Natural products with antiinflammatory activities against autoimmune myocarditis. In *Inflammation and Natural Products*; Nair, A., Gopi, S., Eds.; Academic Press Inc.: London, UK, 2021; pp. 21–38.
14. Li, F.; Chai, R.J.; Fan, Q.L. Supplementing formulas. In *Applications of Chinese Formula Compatibility*; Iong, D., Garran, T.A., Deasy, L., Eds.; People's Medical Publishing House: Beijing, China, 2019; pp. 372–440.
15. Liang, X.; Bi, X.; Kamruzzaman, M.; Sano, H. Effect of Chinese herbal medicine on kinetics of plasma phenylalanine, tyrosine and whole body protein synthesis in sheep. *Anim. Sci. J.* **2019**, *90*, 533–538. [CrossRef] [PubMed]
16. NRC (National Research Council). *Nutrient Requirements of Sheep*, 6th ed.; National Academy Press: Washington, DC, USA, 1985.
17. Weatherburn, M.W. Phenol-hypochlorite reaction for determination of ammonia. *Anal. Chem.* **1967**, *39*, 971–974. [CrossRef]
18. Chen, X.B.; Gomes, M.J. *Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives—An Overview of the Technical Details*; Rowett Research Institute: Aberdeen, UK, 1992; pp. 1–21.
19. Sano, H.; Kajita, M.; Fujita, T. Effect of dietary protein intake on plasma leucine flux, protein synthesis, and degradation in sheep. *Comp. Biochem. Physiol. B* **2004**, *139*, 163–168. [CrossRef]
20. Wolfe, R.R. Tracers in metabolic research: Radioisotope and stable isotope/mass spectrometry methods. *Lab. Res. Methods Biol. Med.* **1984**, *9*, 1–287.
21. Castillo, A.R.; Kebreab, E.; Beever, D.E.; Barbi, J.H.; Sutton, J.D.; Kirby, H.C.; France, J. The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *J. Anim. Sci.* **2001**, *79*, 247–253. [CrossRef]
22. Chao, S.C.; Young, D.G.; Oberg, C.J. Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. *J. Essent. Oil Res.* **2000**, *12*, 639–649. [CrossRef]
23. Calsamiglia, S.; Busquet, M.; Cardozo, P.W.; Castillejos, L.; Ferrt, A. Essential oils as modifiers of rumen microbial fermentation. *J. Dairy Sci.* **2007**, *90*, 2580–2595. [CrossRef]
24. Benchaar, C.; Calsamiglia, S.; Chaves, A.V.; Fraser, G.R.; Colombatto, D.; McAllister, T.A.; Beauchemin, K.A. A review of plant-derived essential oils in ruminant nutrition and production. *Anim. Feed Sci. Technol.* **2008**, *145*, 209–228. [CrossRef]
25. Soltan, M.A.; Shewita, R.S.; Al-Sultan, S.I. Influence of essential oils supplementation on digestion, rumen fermentation, rumen microbial populations and productive performance of dairy cows. *Asian J. Anim. Sci.* **2009**, *3*, 1–12. [CrossRef]
26. Yang, W.Z.; Benchaar, C.; Ametaj, B.N.; Chaves, A.V.; He, M.L.; McAllister, T.A. Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. *J. Dairy Sci.* **2007**, *90*, 5671–5681. [CrossRef] [PubMed]
27. Hart, K.J.; Yáñez-Ruiz, D.R.; Duval, S.M.; McEwan, N.R.; Newbold, C.J. Plant extracts to manipulate rumen fermentation. *Anim. Feed Sci. Technol.* **2008**, *147*, 8–35. [CrossRef]
28. Fujita, T.; Kajita, M.; Sano, H. Responses of whole body protein synthesis, nitrogen retention and glucose kinetics to supplemental starch in goats. *Comp. Biochem. Physiol. B* **2006**, *144*, 180–187. [CrossRef] [PubMed]
29. Wanapat, M.; Boonnop, K.; Promkot, C.; Cherdthong, A. Effects of alternative protein sources on rumen microbes and productivity of dairy cows. *Maejo Int. J. Sci. Technol.* **2011**, *5*, 13–23.
30. Chen, X.B.; Ørskov, E.R.; Hovell, F.D.D. Excretion of purine derivatives by ruminants: Endogenous excretion, differences between cattle and sheep. *Br. J. Nutr.* **1990**, *63*, 121–129. [CrossRef]
31. Chen, X.B.; Chen, Y.K.; Franklin, M.F.; Ørskov, E.R.; Shand, W.J. The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep. *J. Anim. Sci.* **1992**, *70*, 1534–1542. [CrossRef]
32. Kamruzzaman, M.; Liang, X.; Sekiguchi, N.; Sano, H. Effect of feeding garlic leaf on microbial nitrogen supply, kinetics of plasma phenylalanine, tyrosine and protein synthesis in sheep. *Anim. Sci. J.* **2014**, *85*, 542–548. [CrossRef]
33. Castillejos, L.; Calsamiglia, S.; Ferret, A.; Losa, R. Effects of a specific blend of essential oil compounds and the type of diet on rumen microbial fermentation and nutrient flow from a continuous culture system. *Anim. Feed Sci. Technol.* **2005**, *119*, 29–41. [CrossRef]

34. Benchaar, C.; Petit, H.V.; Berthiaume, R.; Ouellet, D.R.; Chiquette, J.; Chouinard, P.Y. Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. *J. Dairy Sci.* **2007**, *90*, 886–897. [CrossRef]
35. Zhong, R.Z.; Yu, M.; Liu, H.W.; Sun, H.X.; Cao, Y.; Zhou, D.W. Effects of dietary *Astragalus* polysaccharide and *Astragalus membranaceus* root supplementation on growth performance, rumen fermentation, immune responses, and antioxidant status of lambs. *Anim. Feed Sci. Technol.* **2012**, *174*, 60–67. [CrossRef]
36. Yin, F.G.; Liu, Y.L.; Yin, Y.L.; Kong, X.F.; Huang, R.L.; Li, T.J.; Wu, G.Y.; Hou, Y. Dietary supplementation with *Astragalus* polysaccharide enhances ileal digestibilities and serum concentrations of amino acids in early weaned piglets. *Amino Acids* **2009**, *37*, 263–270. [CrossRef] [PubMed]
37. Alam, M.K.; Ogata, Y.; Sato, Y.; Sano, H. Effects of rice straw supplemented with urea and molasses on intermediary metabolism of plasma glucose and leucine in sheep. *Asian-Aust. J. Anim. Sci.* **2016**, *29*, 523–529. [CrossRef] [PubMed]
38. Wessels, R.H.; Titgemeyer, E.C.; St Jean, G. Effect of amino acid supplementation on whole-body protein turnover in Holstein steers. *J. Anim. Sci.* **1997**, *75*, 3066–3073. [CrossRef] [PubMed]
39. Magni, F.; Arnoldi, L.; Galati, G.; Kienle, M.G. Simultaneous determination of plasma levels of α-ketoisocaproic acid and leucine and evaluation of α-[1-^{13}C]ketoisocaproic acid and [1-^{13}C]leucine enrichment by gas chromatography-mass spectrometry. *Anal. Biochem.* **1994**, *220*, 308–314. [CrossRef]
40. Li, X.; Wei, W. *Chinese Materia Medica: Combinations and Applications*; Donica Publishing Ltd.: Herts, UK, 2002.
41. Zhang, Q.; Zhu, L.L.; Chen, G.G.; Du, Y. Pharmacokinetics of astragaloside *iv* in beagle dogs. *Eur. J. Drug Metab. Pharmacokinet.* **2007**, *32*, 75–79. [CrossRef]
42. Li, L.; Yu, H.; Pan, J. A study on protein metabolism in nephrotic patients treated with Chinese herbs. *Zhonghua Nei Ke Za Zhi* **1995**, *34*, 670–672.
43. Lapierre, H.; Blouin, J.P.; Bernier, J.F.; Reynolds, C.K.; Dubreuil, P.; Lobley, G.E. Effect of supply of metabolizable protein on whole body and splanchnic leucine metabolism in lactating dairy cows. *J. Dairy Sci.* **2002**, *85*, 2631–2641. [CrossRef]