Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org

Kafkas Univ Vet Fak Derg
28 (2): 177-184, 2022
DOI: 10.9775/kvfd.2021.26647

Research Article

Effects of Supplementation with Rumen-Protected Methionine on Milk Performance, Plasma Biochemical Indices and Amino Acid Concentration in Dairy Goats Subject to Heat Stress

Li LI [1,a] Xinyi LAN [1,b] Hao LING [1,c] Jintao QU [1,2,d]
Qianming JIANG [1,e] Shengguo TANG [1,f] Peihua ZHANG [1,g (*)]

[1] College of Animal Science and Technology, Hunan Agricultural University, Changsha 410128, CHINA

[2] Chengbu Shanmu Animal Husbandry Co.,Ltd., Shaoyang 422500, CHINA

ORCIDs: [a] 0000-0002-8976-6306; [b] 0000-0002-2709-0515; [c] 0000-0001-7885-6720; [d] 0000-0002-0164-9624; [e] 0000-0001-9522-4856
[f] 0000-0003-4011-5796; [g] 0000-0003-1408-122X

Article ID: KVFD-2021-26647 Received: 01.11.2021 Accepted: 17.03.2022 Published Online: 17.03.2022

Abstract: This study examined the effects of four levels of rumen-protected methionine (RPM) on the milk performance of dairy goats subjected to severe heat stress (temperature humidity index >90). Seventy-five Guanzhong dairy goats (52.6±4.9 kg) with the same farrowing period and lactation day (120±10 d) were randomly divided into five groups of 15 head and fed a 13.55% crude protein diet supplemented with 0, 1.5, 3, 4.5 or 6 g/d RPM in the concentrate. The trial included nine days adaptation time and 30 days of sampling and analysis. The addition of RPM did not change feed intake but 1.5 g/d RPM significantly increased milk protein content, the ratio of milk to feed, and the economic returns. No significant changes in milk fat or urea-nitrogen concentrations or somatic cell count were observed. Plasma urea-nitrogen was significantly lowered in the 1.5 g/d RPM group. RPM supplementation increased plasma methionine concentration and total amino acid concentration in a dose-dependent manner. The highest dose of RPM (6 g/d) enhanced plasma immunoglobulin G concentration. It is demonstrated that supplementation with an appropriate dose of RPM to dairy goats fed a relatively low protein diet and subject to heat stress can increase milk protein production and improve economic returns.

Keywords: *Rumen protected methionine, Milk composition, Plasma urea-nitrogen, Free amino acids, Immunoglobulin G, Dairy goats*

Isı Stresine Maruz Kalmış Süt Keçilerinde Rumen Korumalı Metiyonin İlavesinin Süt Performansı, Plazma Biyokimyasal İndeksleri ve Amino Asit Konsantrasyonu Üzerine Etkileri

Öz: Bu çalışmada, şiddetli ısı stresine (sıcaklık-nem indeksi >90) maruz kalan süt keçilerinde rumen korumalı metiyoninin (RPM) dört farklı konsantrasyonunun süt performansı üzerine etkisi incelendi. Aynı yavrulama zamanı ve laktasyon dönemine (120±10 gün) denk gelen 75 Guanzhong süt keçisi (52.6±4.9 kg), 15 hayvandan oluşan beş gruba ayrıldı ve her bir grup 0, 1.5, 3, 4.5 ve 6 g/gün konsantreli RPM içeren %13.55 ham protein diyeti ile beslendi. Deneme, dokuz günlük adaptasyon süresi ve 30 günlük örneklem ve analiz süresini içeriyordu. RPM ilavesi yem tüketimini değiştirmez iken, 1.5 g/gün konsantreli RPM ilavesi süt protein içeriğini, sütün besleme oranını ve ekonomik kazancı önemli ölçüde artırdı. Süt yağı ve üre-azot konsantrasyonlarında ve somatik hücre sayısında önemli bir değişiklik gözlenmedi. 1.5 g/gün konsantreli RPM uygulanan grupta plazma üre-azot seviyesi önemli ölçüde azaldı. RPM ilavesi, doza bağımlı bir şekilde plazma metiyonin ve toplam amino asit konsantrasyonunu arttırdı. RPM'nin en yüksek dozu (6 g/gün), plazma immünoglobulin G konsantrasyonunu artırdı. Nispeten düşük protein diyeti ile beslenen ve ısı stresine maruz kalan süt keçilerine uygun dozlu RPM ilavesinin, süt protein üretimini artırabileceği ve ekonomik kazancı iyileştirebileceği gösterilmiştir.

Anahtar sözcükler: *Rumen koruyucu metiyonin, Süt bileşimi, Plazma üre-azot, Serbest amino asitler, İmmünoglobulin G, Süt keçisi*

Introduction

Low protein utilization and lack of protein-rich feed make ruminant production less economical. It is necessary to improve the utilization of protein by ruminants [1] the limiting of supplemental amino acids being an important approach [2]. Heat stress is a series of non-specific physiological reactions in animals subject to high

How to cite this article?

Li L, Lan X, Ling H, Qu J, Jiang Q, Tang S, Zhang P: Effects of supplementation with rumen-protected methionine on milk performance, plasma biochemical indices and amino acid concentration in dairy goats subject to heat stress. *Kafkas Univ Vet Fak Derg,* 28 (2): 177-184, 2022.
DOI: 10.9775/kvfd.2021.26647

(*) Corresponding Author

Tel: +86-13548638664
E-mail: zhang1970@hunau.edu.cn (P. Zhang)

temperature and high humidity environments, and is an important factor hindering the development of the dairy goat industry due to its adverse effects on milk production and composition [3-5]. Studies have shown that when animals are subjected to heat stress their feed intake, reproductive performance, and productivity are affected, and, in severe cases, death can result [6]. Heat stress affects immune function at the interface of the endocrine and immune systems. Lactating ewes under heat stress have shown elevated mastitis pathogen loads [7]. Thus, the alleviation of heat stress and improvement of ruminant performance must be urgently addressed.

Methionine (Met) is considered to be the primary limiting amino acid in milk production in dairy ruminants [8,9]. It is an effective regulator of protein synthesis [10,11] and involved in transsulfuration, methylation reactions and polyamine synthesis [11]. Met has a significant impact on oxidative stress status because it is essential for the synthesis of glutathione, one of the most abundant antioxidants produced in the liver [12]. So, the essential amino acid methionine is one of the antioxidant nutrients that act to mitigate the deleterious effects of ROS to cells. Thus, in addition to involvement in glutathione synthesis, Met supplementation has a direct protective effect against oxidative stress in animals under heat stress. Its functions include the maintenance of animal growth, the development of physiological activities, detoxification and anti-mould activities, and myocardial protection. Insufficient Met can cause animal weight loss, stunted growth, liver and kidney dysfunctions, and poor milk quality [13].

Dietary Met can be rapidly degraded by microorganisms in the rumen, while Met in the microbial proteins that enter the small intestine of the host is usually insufficient for high milk production [14]. Previous studies have shown that supplementing the feed of lactating ruminants with rumen-protected methionine (RPM) consistently increased milk protein concentration and milk protein yield [15-18]. Supplementation of dairy cow diets with RPM can improve the utilization efficiency of feed protein, fulfil protein requirements for lactation and reduce the influence of heat stress on milk yield. Zhao et al.[19] compared a low-protein diet (12% CP) supplemented with RPM to a high-protein diet (16% CP). The supplemented low-protein diet increased both milk protein yield and urea-nitrogen content and tended to increase milk yield. Lee et al.[20] reduced the dietary protein of dairy cows from 15.7% to 13.5% and found a significant reduction in milk yield that could compensated by supplementation with RPM. Many reports have revealed that supplementation with RPM increased milk production and immunity of lactating cows [21,22], but there have been few studies on RPM supplementation of dairy goats.

The main hypothesis of this study is that adding RPM to a low-protein diet increases plasma Met concentrations, promotes lactation, improves the utilization of dietary protein by dairy goats, and alleviates the effects of heat stress. The National Research Council (NRC) [23] state that it is common to supplement dairy goats with Met, but the optimal dose of RPM is unknown. In this study various doses of RPM were given to dairy goats and the effects on milk quantity and quality and blood indices were assessed, in order to define the appropriate RPM dose for dairy goats subject to heat stress.

Material and Methods

Source of RPM

RPM containing 57% 2-hydroxy-4-(methylthio)isopropyl butyrate was purchased from Adisseo Life Science Products Co., Ltd. (Shanghai, China).

Animals and Experimental Design

The trial was conducted at Taiping Farm (Shaoyang, Hunan, China; latitude 27°14'N, longitude 111°27'E). Seventy-five Guanzhong dairy goats, a local Chinese breed, with similar live weights (52.6±4.9 kg) and at similar lactation ages (120±10 d) were selected and allocated to five group consisting of 15 head per group. All animals were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the College of Animal Science and Technology, Hunan Agricultural University (CASTHAU-02-2019-10). The groups were randomly assigned to five treatments involving supplementation of the basal diet with RPM at doses of 0 (control group), 1.5, 3.0, 4.5, and 6.0 g/d head. The trial lasted for 39 days, consisting of nine days for acclimation and 30 days (the experimental period) for sampling and analyses.

Diet and Animal Management

The diet referenced NRC nutrient requirements for dairy goats [23] and was prepared as a total mixed ration. Dietary ingredients and nutritional parameters are listed in *Table 1*. The average CP content of the basal diet in this study (13.55%) was lower than the CP content recommended by the NRC [23]. Diet with low protein level (13.55%) was fed to groups in the current study. Goats were kept in individual pens and fed twice daily at 08:00 and 18:00. Fresh drinking water was available all times.

Measurement of Temperature-Humidity Index (THI)

Wet-bulb and dry-bulb thermometers were hung in the barn 1.5 m above the ground. Temperature and humidity were recorded every day at 08:00, 12:00, and 17:00. The formula THI = 0.72 x (Td + Tw) + 40.6 was applied, where Td represents dry-bulb temperature and TW represents wet-bulb temperature [24]. The average of the two thermometer readings was recorded. As shown in

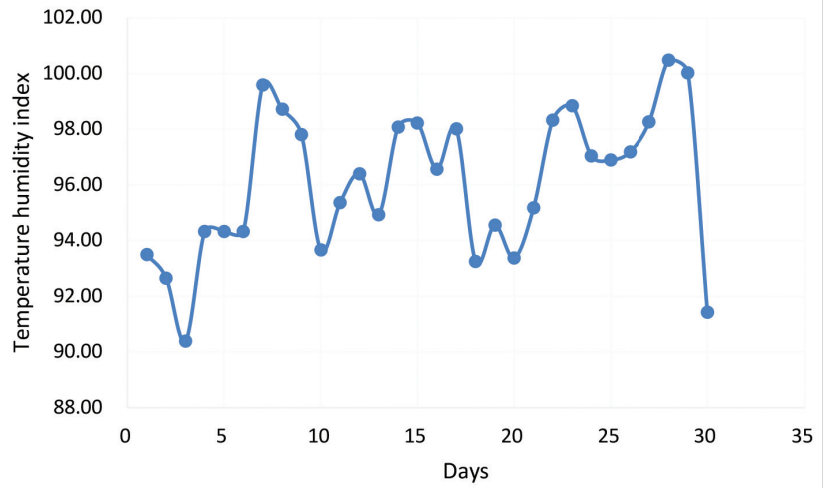

Fig 1. The temperature-humidity index figure during the experimental period

Table 1. *Composition and nutritional parameters of the basal diet (dry matter basis)*

Ingredients	%	Nutritional Parameters	%
Alfalfa hay	24.00	DM	92.94
Oat hay	24.00	CP	13.55
Alfalfa pellets	12.00	NDF	52.75
Corn	27.72	ADF	21.96
Wheat middling	2.00	Ca	0.39
Soybean meal	9.30	P	0.26
$Ca(HCO_3)_2$	0.60	Met	0.24
NaCl	0.30		
$CaCO_3$	0.03		
Premix[1]	0.05		
Total	100.00		

[1] Premix provides (Purina commercial feed, per kg feed, dry matter basis): Vit. A- 15.544 IU, Vit. D- 23.220 IU, Vit. E- 297 IU, Fe- 30 mg, Cu- 25 mg, Zn- 60 mg, Mn- 75 mg, I- 0.15 mg, Se- 0.05 mg, Co- 0.15 mg
DM: *dry matter;* ***CP:*** *crude protein;* ***NDF:*** *neutral detergent fiber;* ***ADF:*** *acid detergent fiber;* ***Ca:*** *calcium;* ***P:*** *phosphorus;* ***Met:*** *methionine*

Fig. 1, the THI during the experimental period was >90, indicating the animals were subject to severe heat stress [25].

Sampling and Analyses

The quantities of feed offered and refused were recorded daily during the experimental period to calculate the average daily feed intake. Samples of the ration and any feed refused were taken daily and stored at -20°C. At the end of the experimental period, feed samples were pooled, subsampled, and dried at 65°C for 48 h. Dried samples were ground through a 40-mesh sieve and their chemical composition determined using methods described by the Association of Official Analytical Chemists [26].

Goats were milked twice per day during the experimental period using a parallel milking machine (DeLaval Co., Ltd., Sweden) and milk yield was recorded. Milk samples (200 mL stored at -20°C) were taken on the last day from nine randomly selected goats in each group. These were aliquoted into 4 x 50 mL centrifuge tubes for milk composition determination. There were three replicates per sample. Concentrations of fat, protein, and non-fat milk solids were determined using a MILKANA Milk Analyzer (Beijing Harold Technology Co., Ltd., China). Somatic cell count (SCC) was determined using a Lilavar SCC analyzer (Nova Zagora, Bulgaria). Urea-nitrogen was determined with an enzymatic (urease) method using a commercial kit (Nanjing Jiancheng, China) following the manufacturer's protocol.

Feed costs, gross income from milk, and economic return from the milk (i.e., ratio of milk income to the feed cost) were calculated as follows:

Feed costs (¥/d) = Feed intake (kg/d) x Feed price (¥1.68/kg) + RPM cost (¥75/kg)

Milk income (¥/d) = Milk yield (kg/d) x Milk price (¥7.50/kg)

Gross milk income (¥/d) = Milk income - Feed cost

where ¥ is Renminbi (RMB). The ratio of average milk yield (kg/d) to average feed intake (kg/d) was also calculated.

On day 30 of the experimental period, nine goats from each group were randomly selected for blood sampling before morning feeding. A total of 15 mL of blood was drawn from the jugular vein and plasma was obtained by centrifugation at 1.600 x g for 20 min and stored at -20°C. Plasma concentrations of urea-nitrogen, glucose, triglycerides, total cholesterol, and immunoglobulin G were determined using commercial kits (Nanjing Jiancheng Biotechnology Co., Ltd., Nanjing, China) following the manufacturer's procedures. Free amino acid concentrations were determined by a high-performance liquid chromatography (LC-10ADvp equipped with Fr-10ADvp; Shimadzu, Japan) according to the method of Hughes et al.[27].

Statistical Analysis

Differences between the five trial groups were analyzed by one-way ANOVA using SAS 9.4 software [28]. Since four doses of RPM were offered, the linear, quadratic and cubic trends were tested using CONTRAST statements with orthogonal polynomial coefficients estimated by PROC IML. Data are presented as least square means and standard error of the means. Differences between means with P≤ 0.05 were considered statistically significant.

Results

Effects of RPM on Milk Performance and Economic Return

As shown in *Table 2,* there were no significant differences in dry matter intake or milk yield between the groups (P>0.05). However, milk yield showed a significant quadratic increase (P<0.05) in the treatment groups compared with the control group. The milk to feed ratio was higher in the 1.5, 3, and 4.5 g/d RPM groups than in the control and 6 g/d RPM groups (P<0.05) while quadratic and cubic effects were evident (P<0.05).

Protein and non-fat solids concentrations were highest in the 6 g/d RPM group, followed by the 1.5 g/d RPM group. Both concentrations were significantly higher in these groups than in the other three groups (P<0.05), and exhibited significant cubic responses (P<0.05). No significant differences between groups were found for milk fat and urea-nitrogen concentrations and SCC count (P>0.05), but milk fat in RPM-supplemented groups was higher than in the control group.

There were no significant differences in milk income among groups (P>0.05). However, milk income showed a significant quadratic trend (P<0.05), being higher in the treatment groups than the control group. Gross profit showed linear, quadratic and cubic relationships with RPM dose and was higher in the 1.5 and 3 g/d RPM groups than in the other groups (P<0.05). Feed cost increased with the quantity of RPM supplement and displayed linear, quadratic, and cubic relationships (P<0.05).

Effects of RPM on Plasma Biochemical Parameters

Plasma urea-nitrogen concentration was lower in the treatment groups than the control group, with the 1.5 g/d group being significantly lower than other treatment groups (P<0.05). There were no significant differences between the groups in plasma concentrations of glucose, non-esterified fatty acids, total cholesterol, and total triglycerides (P<0.05; *Table 3),* but total cholesterol and total glycerides were lower in the treatment groups than in the control group. Immunoglobulin G was highest in the 6 g/d RPM group (P<0.05), and showed linear, quadratic, and cubic relationships (P<0.05).

Effects of RPM on Plasma Free Amino Acid Concentration

Table 4 shows plasma concentrations of essential and non-essential amino acids. Supplementation with RPM increased Met concentration in a dose-dependent manner, the increments appearing between the 0 to 4.5 g/d RPM groups but being much greater in the 6 g/d group.

Generally, RPM supplementation increased the total essential amino acid (TEAA) concentration in plasma, but this effect varied between RPM doses. The greatest

Table 2. *Effects of supplementation with rumen-protected methionine on milk production and economic returns in dairy goats*

Item	Rumen-Protected Methionine Supplement (g/d)					SEM	P-value		
	0	1.5	3	4.5	6		Linear	Quadratic	Cubic
DMI, kg/d	2.65	2.64	2.70	2.63	2.67	0.021	0.607	0.774	0.791
Milk yield, kg/d	0.79	0.85	0.87	0.84	0.80	0.030	0.835	0.048	0.107
Milk-feed ratio	0.30^{a}	0.32^{b}	0.32^{b}	0.32^{b}	0.30^{a}	0.003	0.666	<0.001	<0.001
Milk Composition									
Fat, %	3.11	3.26	3.43	3.70	3.27	0.184	0.230	0.159	0.173
Protein, %	3.05^{a}	3.20^{b}	3.06^{a}	3.07^{a}	3.21^{b}	0.048	0.252	0.409	0.032
SNF,%	8.07^{a}	8.46^{b}	8.09^{a}	8.10^{a}	8.51^{b}	0.134	0.293	0.445	0.036
MUN, mmol/L	3.21	3.34	3.55	3.86	3.28	0.184	0.400	0.220	0.184
SCC, ×10^{4}/mL	23.84	24.41	24.25	39.48	29.67	0.353	0.247	0.514	0.504
Economic Returns									
Milk income, ¥/head·d	5.55	5.93	6.07	5.86	5.58	0.212	0.865	0.048	0.107
Feed cost, ¥/head·d	4.44^{a}	4.56^{b}	4.76^{c}	4.76^{c}	4.94^{d}	0.036	<0.001	<0.001	<0.001
Gross profit, ¥/head·d	1.11^{b}	1.37^{c}	1.31^{c}	1.10^{b}	0.64^{a}	0.035	<0.001	<0.001	<0.001

DMI: *dry matter intake;* ***Milk income:*** *milk yield x milk price;* ***MUN:*** *milk urea-nitrogen;* ***SCC:*** *somatic cell count;* ***SNF:*** *non-fat milk solids;* ***¥:*** *Chinese Renminbi; Mean values within a row with different superscript letters differ significantly (P<0.05)*

Table 3. Effects of supplementation with rumen-protected methionine on plasma biochemical indices in dairy goats

Item	Rumen-Protected Methionine Supplement (g/d)					SEM	P-value		
	0	1.5	3	4.5	6		Linear	Quadratic	Cubic
PUN, mmol/L	6.31[b]	5.08[a]	6.09[b]	5.91[b]	6.03[b]	0.264	0.861	0.647	0.423
GLU, mmol/L	1.83	1.60	1.67	1.64	1.62	0.141	0.448	0.667	0.774
IgG, mg/mL	9.42[a]	9.24[a]	9.31[a]	9.26[a]	10.69[b]	0.243	0.048	0.014	0.023
NEFA, mmol/L	0.25	0.22	0.27	0.25	0.23	0.021	0.929	0.781	0.586
TC, mmol/L	2.99	2.57	2.76	2.58	2.46	0.145	0.068	0.187	0.234
TG, mmol/L	0.30	0.22	0.29	0.24	0.28	0.023	0.997	0.384	0.486

PUN: *plasma urea-nitrogen;* ***GLU:*** *glucose;* ***IgG:*** *immunoglobulin G;* ***NEFA:*** *non-esterified fatty acids;* ***TC:*** *total cholesterol;* ***TG:*** *triglycerides; Mean values within a row with different superscript letters differ significantly (P<0.05)*

Table 4. Effects of supplementation with rumen-protected methionine on plasma free amino acid concentrations (mg/100 mL) in dairy goats

Item	Rumen-Protected Methionine Supplement (g/d)					SEM	P-value		
	0	1.5	3	4.5	6		Linear	Quadratic	Cubic
EAA									
Met	0.37[a]	0.51[b]	0.67[c]	0.84[d]	1.40[e]	0.050	<0.001	<0.001	<0.001
Lys	1.72[a]	2.12[b]	2.54[c]	2.12[b]	2.12[b]	0.120	0.009	0.006	0.012
Leu	1.98[a]	1.85[a]	2.29[a]	2.16[a]	3.67[b]	0.150	<0.001	<0.001	<0.001
Ile	1.78[a]	1.35[a]	2.65[b]	2.69[b]	4.84[c]	0.175	<0.001	<0.001	<0.001
His	0.70[a]	0.83[ab]	1.37[c]	0.96[b]	1.45[c]	0.079	<0.001	<0.001	<0.001
Arg	2.72	3.00	2.94	2.52	2.76	0.153	0.560	0.633	0.256
Phe	1.30[a]	1.62[a]	2.25[b]	2.28[b]	2.46[b]	0.110	<0.001	<0.001	<0.001
Thr	5.68[a]	7.38[b]	9.25[d]	6.88[b]	8.27[c]	0.248	0.004	0.001	<0.001
Val	3.62[a]	4.22[a]	5.46[b]	5.66[b]	8.38[c]	0.220	<0.001	<0.001	<0.001
BCAA	7.58[b]	7.11[a]	10.58[c]	10.29[c]	17.90[d]	0.110	<0.001	<0.001	<0.001
TEAA	20.04[a]	22.20[b]	30.91[d]	25.06[c]	37.28[e]	0.324	<0.001	<0.001	<0.001
NEAA									
Ala	2.87[a]	3.27[a]	5.88[b]	6.19[b]	10.92[c]	0.215	<0.001	<0.001	<0.001
Asp	0.15	0.12	0.14	0.14	0.15	0.015	0.601	0.592	0.689
Glu	2.60[a]	2.44[a]	3.45[b]	2.82[a]	3.88[b]	0.162	<0.001	0.001	0.003
Ser	1.00[a]	1.11[a]	1.83[b]	1.71[b]	2.73[c]	0.104	<0.001	<0.001	<0.001
Gly	0.67[a]	1.83[b]	2.39[c]	2.06[b]	2.45[c]	0.106	<0.001	<0.001	<0.001
Tyr	0.79[a]	1.44[b]	2.19[d]	1.79[c]	2.12[d]	0.105	<0.001	<0.001	<0.001
NEAA	8.07[a]	10.20[b]	16.07[c]	16.22[c]	24.70[d]	0.230	<0.001	<0.001	<0.001
TAA	28.11[a]	32.29[a]	46.99[c]	38.27[b]	59.60[d]	1.905	<0.001	<0.001	<0.001

Ala: *alanine;* ***Arg:*** *arginine;* ***Asp:*** *aspartic acid;* ***BCAA:*** *branched chain amino acids;* ***Glu:*** *glutamate;* ***Gly:*** *glycine;* ***His:*** *histidine;* ***Ile:*** *isoleucine;* ***Leu:*** *leucine;* ***Lys:*** *lysine;* ***Met:*** *methionine;* ***NEAA:*** *non-essential amino acids;* ***Phe:*** *phenylalanine;* ***Ser:*** *serine;* ***TAA:*** *total amino acids;* ***TEAA:*** *total essential amino acids;* ***Thr:*** *threonine;* ***Tyr:*** *tyrosine;* ***Val:*** *valine; Mean values within a row with different superscript letters differ significantly (P<0.05).*

TEAA increment was with the 6 g/d RPM group, followed by the 3 g/d group, then the 4.5 and 1.5 g/d groups *(Table 4)*. Branched-chain amino acids (BCAA) were lowered by supplementation with 1.5 g/d RPM but were significantly increased in the other treatment groups. The effects of RPM supplementation on individual EAAs varied, depending on RPM dose. In general, RPM supplementation increased plasma concentrations of Lys, His, Phe, and Thr, but had no significant influence on Arg concentration.

Supplementation with RPM had no significant influence on Asp concentration in plasma (P>0.05) but increased the concentration of the other non-EAAs (Ala, Glu, Ser, Gly, and Tyr). The total concentration of non-EAAs increased with RPM dose *(Table 4)*.

Total amino acid concentration increased linearly with RPM dose, except for the 4.5 g/d RPM group which was lower than the 3 and 6 g/d groups. In addition, linear, quadratic, and cubic relationships were evident (P<0.05) with Met, Lys, Ile, His, Phe, Thr, Val, BCAA, EAA, Ala, Gly, Ser, Gly, and Tyr, and non-EAA.

Discussion

Goats living under conditions of heat stress with 13.55% crude protein in their diets (i.e., a low-protein diet) produced 0.8-0.9 kg of milk per day. Adding 1.5 or 6 g/d RPM to the diet increased the protein and non-fat solid contents of the milk and improved economic returns. Milk yield showed a significant quadratic increase, being higher in the treatment groups than the control group. Milk fat concentration tended to increase in response to RPM dosage (1.5 to 4.5 g/d) but did not reach statistical significance. The present findings are supported by previous reports. Mateus et al.[29] showed that cows given RPM had increased milk protein (3.07 vs. 2.95%), yield (1.48 vs. 1.43 kg/d), and fat (3.87 vs. 3.77%), although milk fat yield did not change. Similar increases in protein yield and concentration following RPM supplementation have been reported in dairy goats. For example, a 2.5 g/d RPM supplement given to Shami goats from the final 60 days of pregnancy through the first 60 days of lactation was reported to increase milk yield from 1.18 kg/d (control group) to 1.36 kg/d, and milk protein concentration from 3.55% to 3.85%. The Shami goats weighed 47-75 kg and were fed a basal diet containing 17.5% crude protein [30]. In Zaraibe goats (bodyweight ~35 kg) fed a basal diet containing about 14% crude protein, supplementation with 2 g/d RPM increased milk yield from 1.73 kg/d (control group) to 2.07 kg/d. Milk protein increased from 8.67% to 9.24% (RPM group), respectively, SNF also increased correspondingly [31]. Increments in milk yield and protein concentration were also observed in Saanen goats fed a basal diet containing 14.4% crude protein and supplemented with 2.5 g/d RPM [32], and in Alpine dairy goats supplemented with 5 g/d RPM [33]. These effects vary depending on lactation stage, milk yield and dietary crude protein content [13]. Heat stress adversely affects milk production and composition in dairy animals. According to Bouraoui et al.[34], daily THI negatively correlated to milk yield, and lower milk fat and milk protein were observed in the summer season. Hamzaoui et al.[35] also reported milk with lower protein (6-13%) content in heat stressed goats. This demonstrates that heat stress affects the health of dairy animals, impacting their normal physiology and metabolism. The results of this study show that RPM supplementation tends to improve milk yield in dairy goats, possibly because it increases Met supply in the small intestine, and, subsequently, the mammary gland, providing sufficient precursors for protein synthesis in the gland. Thus, RPM supplementation effectively promotes the metabolic balance of amino acids in ruminants, improves the utilization rate of amino acids, and reduces the adverse effects of heat stress, providing adequate energy for milk production and milk protein synthesis, and promoting performance. In summary, supplementation of low-protein diets with RPM alleviates the negative effects of heat stress. Higher doses of RPM yield no further benefits.

The high observed milk to feed intake ratio and the static feed intake in the 1.5 g/d RPM group suggests that the increases in milk protein and non-fat solids resulted from the increased utilization of nutrients, particularly protein. This study did not measure the digestion and metabolism of N in the goats. However, RPM supplementation may increase protein digestibility in some cases, for example in Zaraibe goats [31]. Muramatsu et al.[36] showed that giving RPM to Japanese Saanen goats substantially increased whole-body protein synthesis while reducing urinary N excretion; so N balance and utilization efficiency were markedly increased at a constant N intake [37]. Improved N utilization efficiency in response to RPM supplementation is supported by the reduced plasma urea-nitrogen concentration observed in the current study, plasma urea-nitrogen being a good indicator of protein status [38].

RPM did not alter the plasma concentrations of glucose, non-esterified fatty acids, total cholesterol, or total glycerides, but total cholesterol and total glycerides in the treatment groups were lower than those in the control group. Total cholesterol content reflects the lipid metabolism status of the body. Total glycerides is a product of fat metabolism and reflects the digestion and absorption status of fat-the higher the content, the lower the utilization rate of body fat [39]. This also corresponded with the milk fat of the treatment groups being higher than the control group. The only substantial change was plasma IgG concentration, which was significantly elevated in the 6 g/d RPM group. The immune system is the major body defense systems to protect and cope against environmental stressors [40]. Met participates in the synthesis of immune system molecules (cytokines, antibodies, complement, etc.) [41] and influences the levels of immunoglobulins in the body. In order to enhance the humoral immune response, Met is present at much higher concentrations than required for production and health needs [42]. The health implications for dairy goats of having such high IgG levels in plasma are not clear and the molecular mechanism by which high levels of Met boost IgG concentrations warrants further research.

Plasma concentrations of free amino acids reflect the balance between amino acids entering the metabolic pool from both intestinal absorption and metabolic protein breakdown, and those leaving the pool as a result of protein synthesis and irreversible oxidation [43]. Based only on changes in plasma concentration, it is not possible to judge how this two-way flux of amino acids contributes to shifts in concentration. It is notable that plasma Met concentration increased in response to RPM dose, suggesting that the amount of Met absorbed from the intestine increased as a result of the supplementation, improving lactation

performance and levels of immunity. Dose-responsive changes in plasma Met concentration have been observed in other studies. For example, plasma free Met and the ratio of Met to total essential amino acids showed significant linear responses to RPM supplementation of Merion sheep (1, 2, 3, 5, and 8 g/d) [44,45] and dairy cows [46]. Literature on changes in plasma Met concentrations in dairy goats supplemented with RPM is scarce, so definitive conclusions cannot be drawn. Increases in total EAA and non-EAA plasma concentrations were observed in this study after RPM supplementation. Changes in total plasma amino acids after RPM supplementation of dairy goats and cows vary. No changes [45,46], decreases [47], and increases [48,49] have all been reported. Amino acids not only serve as direct precursors for protein synthesis, but also act as regulators of the rate of protein synthesis. When activated by Met, mammalian target of rapamycin may increase the initiation rate of protein synthesis [50], which might explain the increased milk protein production of RPM-supplemented dairy goats.

The experimental design of the current study did not allow the source of the increase in total plasma amino acids to be defined; it could be attributed to either increased dietary protein digestion or reduced cellular oxidation of amino acids. Further studies are needed to explore the mechanisms of Met-associated metabolic processes.

In conclusion, supplementing the diet of dairy goats with an appropriate dose of RPM (1.5 g/d) while being fed a relatively low protein diet and subjected to severe heat stress increased milk protein content and improved economic returns, while reducing blood urea-nitrogen. Supplying additional RPM yielded no further production or economic benefits.

Availability of Data and Materials

The authors declare that data supporting the study findings are also available to the corresponding author (P. Zhang).

Acknowledgements

The authors would like to express their gratitude to EditSprings (https://www.editsprings.cn/) for the expert linguistic services provided.

Funding Support

This research was funded by the Key Research and Development Plan of Hunan Province, China (Project No._2016NK2171).

Competing Interest

The authors declare that they have no competing interests.

Author Contributions

P. Zhang designed the study. L. Li drafted and wrote the manuscript. H. Ling collected and analyzed the data. L. Li and J. Qu performed the animal trial and laboratory analysis. Q. Jiang, S. Tang and X. Lan revised the manuscript. All authors gave intellectual input to the study and approved the final version of the manuscript.

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