Effects of Rumen-Protected Tryptophan Supplementation on Plasma Tryptophan and Some Related Metabolites in Sheep: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with rumen-protected tryptophan (RPTrp) on plasma tryptophan (Trp) and related metabolite contents in sheep. Fifteen healthy Suffolk sheep aged (3.0±0.5) years with an average body weight of (53.49±2.41) kg were randomly divided into 3 groups (n=5 per group): a control group and experimental groups I and II. The concentrate feed allowance was 10 g/(kg BW · d), corn silage was 1.8 kg/d, and mixed hay was provided ad libitum. Based on this feeding regimen, sheep in experimental groups I and II were supplemented with 150 mg/(kg BW · d) Trp and 333 mg/(kg BW • d) RPTrp, respectively, for a 25-day feeding trial. The results showed: 1) At 2 h post-feeding in both morning and afternoon, plasma total tryptophan (T-Trp) and free tryptophan (F-Trp) contents in experimental group I were extremely significantly higher than those in the control group ($P < 0.01$), and plasma kynurenine (Kyn) content was significantly higher than that in the control group ($P < 0.05$); at 6 h post morning feeding, plasma T-Trp and F-Trp contents in experimental group II were significantly higher than those in both the control group and experimental group I ($P < 0.05$); at 4 h post afternoon feeding, plasma F-Trp contents in the experimental groups were significantly higher than that in the control group ($P < 0.05$). 2) At 10 h post morning feeding and 8 h post afternoon feeding, plasma 5-hydroxytryptamine (5-HT) content in experimental group II was significantly higher than that in the control group ($P < 0.05$); at 6 h and 8 h post morning feeding, plasma melatonin (ML) contents in the experimental groups were extremely significantly higher than that in the control group ($P < 0.01$), with no significant differences between experimental groups ($P > 0.05$); at 4 h and 8 h post afternoon feeding, experimental group II was significantly higher than the control group ($P < 0.05$). 3) At 2 h post afternoon feeding, plasma free fatty acid (FFA) contents in the experimental groups were extremely significantly lower than that in the

control group ($P < 0.01$); plasma albumin (ALB) contents showed no significant differences among groups at any time point ($P > 0.05$). 4) Compared with the control group, the experimental groups could extremely significantly increase plasma glutathione peroxidase (GSH-Px) activity ($P < 0.05$) and extremely significantly decrease plasma malondialdehyde (MDA) content ($P < 0.01$), and experimental group II could also extremely significantly increase plasma total antioxidant capacity (T-AOC) ($P < 0.01$). Therefore, Trp supplementation could rapidly increase plasma T-Trp, F-Trp, and Kyn contents after feeding in sheep, whereas these effects were more moderate when RPTrp was supplemented. Trp and RPTrp supplementation had minor effects on plasma ALB, FFA, and 5-HT contents in sheep, with significant effects only at individual time points. Trp and RPTrp supplementation increased plasma ML content in sheep during daytime, Trp supplementation had no significant effect on plasma ML content in sheep at night, while RPTrp supplementation could maintain plasma ML content at a relatively high level during nighttime. Trp and RPTrp supplementation could improve plasma antioxidant capacity in sheep.

Full Text

Effects of Rumen-Protected Tryptophan Supplementation on Plasma Tryptophan and Related Metabolites in Sheep

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Abstract

This study investigated the effects of dietary supplementation with rumen-protected tryptophan (RPTrp) on plasma tryptophan (Trp) and related metabolites in sheep. Fifteen healthy Suffolk sheep aged (3.0±0.5) years with an average body weight of (53.49±2.41) kg were randomly allocated into three groups (n=5 per group): a control group, trial group I, and trial group II. All animals received concentrate at 10 g/(kg BW·d), corn silage at 1.8 kg/d, and ad libitum access to mixed hay. Trial groups I and II were additionally supplemented with 150 mg/(kg BW·d) Trp and 333 mg/(kg BW·d) RPTrp, respectively, for a 25-day feeding trial.

The results showed: (1) At 2 h post-feeding in both morning and afternoon, plasma total tryptophan (T-Trp) and free tryptophan (F-Trp) in trial group I were significantly higher than in the control group ($P<0.01$), while plasma kynurenine (Kyn) was significantly elevated ($P<0.05$). At 6 h after morning feeding, plasma T-Trp and F-Trp in trial group II were significantly higher than in both the control and trial group I ($P<0.05$). At 4 h after afternoon

feeding, plasma F-Trp in both trial groups was significantly higher than in the control group ($P<0.05$). (2) At 10 h after morning feeding and 8 h after afternoon feeding, plasma 5-hydroxytryptamine (5-HT) in trial group II was significantly higher than in the control group ($P<0.05$). At 6 and 8 h after morning feeding, plasma melatonin (ML) in both trial groups was significantly higher than in the control group ($P<0.01$), with no significant difference between trial groups ($P>0.05$). At 4 and 8 h after afternoon feeding, plasma ML in trial group II was significantly higher than in the control group ($P<0.05$). (3) At 2 h after afternoon feeding, plasma free fatty acids (FFA) in both trial groups were significantly lower than in the control group ($P<0.01$). No significant differences in plasma albumin (ALB) were observed among groups at any time point ($P>0.05$). (4) Compared with the control group, both trial groups showed significantly increased plasma glutathione peroxidase (GSH-Px) activity and decreased malondialdehyde (MDA) content ($P<0.01$), while trial group II also exhibited significantly elevated total antioxidant capacity (T-AOC) ($P<0.01$).

In conclusion, Trp supplementation rapidly increased plasma T-Trp, F-Trp, and Kyn concentrations post-feeding, whereas RPTrp produced more moderate effects. Both Trp and RPTrp had minimal impacts on plasma ALB, FFA, and 5-HT, with significant effects only at isolated time points. Supplementation increased daytime plasma ML concentrations; Trp had no significant effect on nighttime ML, while RPTrp maintained nighttime plasma ML at relatively elevated levels. Both supplements enhanced plasma antioxidant capacity.

Keywords: sheep; rumen-protected tryptophan; free tryptophan; kynurenine; 5-hydroxytryptamine; melatonin

Introduction

Melatonin (ML) is an indole hormone synthesized and secreted primarily by the pineal gland and intestinal mucosa in mammals, with widespread distribution across numerous organs, tissues, and cells. Research demonstrates that ML promotes oocyte maturation, maintains sperm function, enhances embryonic development, and improves antioxidant capacity in sheep. Therefore, increasing endogenous ML concentrations may have important implications for reproductive performance and antioxidant status.

Tryptophan (Trp) is an essential functional amino acid and precursor for ML synthesis, undergoing hydroxylation, decarboxylation, acetylation, and methylation to form ML. Huenther et al. confirmed that dietary supplementation or intraperitoneal injection of 150-300 mg/(kg BW · d) Trp significantly increased plasma ML concentrations in rats and chickens within 1 h, exhibiting dose-dependent effects. However, in ruminants, Trp is readily degraded by rumen microorganisms into indole, indoleacetic acid, and skatole, limiting the efficacy of direct supplementation. Rumen-protected tryptophan (RPTrp) effectively reduces ruminal degradation. Studies show that supplementing Liaoning cash-

mere goats with 6 g/(head · d) RPTrp (33% Trp content) significantly increased plasma Trp concentrations on days 30, 60, and 90. Our previous research in dairy cows also demonstrated that RPTrp supplementation elevated plasma Trp and nighttime ML concentrations. However, early studies found that intraperitoneal injection of 500 mg/(kg BW · d) Trp did not increase plasma ML in sheep. To further investigate whether increasing intestinal Trp absorption can modulate ML concentrations, this study used sheep as a model to examine the effects of dietary Trp and RPTrp supplementation on plasma concentrations of Trp and its major metabolites, including 5-hydroxytryptamine (5-HT), kynurenine (Kyn), and ML.

Materials and Methods

1.1 Experimental Period and Location The trial was conducted from June to July 2017 at Xinjiang Huikang Animal Husbandry Biotechnology Co., Ltd. Sheep Farm under natural lighting conditions. On sampling days, sunrise occurred at 06:37 and sunset at 21:54, with a photoperiod of 15.27 h.

1.2 Experimental Animals Fifteen healthy Suffolk sheep aged (3.0±0.5) years with an average body weight of (53.49±2.41) kg were selected.

1.3 Experimental Design Animals were randomly assigned to three groups (n=5 per group) based on body weight: control, trial group I, and trial group II. All sheep received the same concentrate (purchased from Xinjiang Tiankang Animal Husbandry Biotechnology Co., Ltd.) at 10 g/(kg BW · d), corn silage at 1.8 kg/d, and ad libitum access to mixed hay (alfalfa:wheat straw = 1:1) and water. Trial group I received 150 mg/(kg BW · d) Trp (L-Trp, purchased from CJ CheilJedang, Indonesia), while trial group II received 333 mg/(kg BW · d) RPTrp (L-RPTrp, purchased from Beijing Yahe Nutrition High-tech Co., Ltd., 45% Trp content, 85% rumen bypass rate). Both trial groups received equal daily Trp intake, with supplementation levels based on Itabashi et al. The diet composition and nutrient levels are presented in Table 1 .

Table 1 Composition and nutrient levels of experimental diets (DM basis)

Items	Concentrate[1])	Corn silage	Alfalfa hay	Wheat straw	Total
Nutrient levels[2])					
Dry matter					

Items	Concentrate[1])	Corn silage	Alfalfa hay	Wheat straw	Total
Crude ash					
Crude protein					
Neutral detergent fiber					
Acid detergent fiber					
Tryptophan					

[1]) Each kg of concentrate contained: corn 0.44 kg, oat 0.16 kg, barley 0.15 kg, soybean meal 0.20 kg, CaHPO 0.03 kg, NaCl 0.01 kg, premix 0.01 kg. The premix provided per kg of concentrate: VA 480 IU, VB 816 mg, VB 333 mg, VB 49 mg, VD 70 IU, VE 21,333 IU, pantothenic acid 20 mg, nicotinamide 485 mg, Cu (as copper sulfate) 11 mg, Fe (as ferrous sulfate) 35 mg, Mn (as manganese sulfate) 33 mg, Zn (as zinc sulfate) 31 mg, I (as potassium iodide) 2 mg, Se (as sodium selenite) 6 mg, Co (as cobalt chloride) 1 mg.

[2]) Trp was a calculated value, while other nutrient levels were measured values.

1.4 Feeding Management Sheep were housed individually. Daily allotments of Trp, RPTrp, concentrate, and corn silage were divided equally and fed at 08:00 and 20:00. To ensure complete consumption, Trp or RPTrp was mixed with 50 g concentrate before feeding; remaining concentrate and corn silage were offered after consumption of the supplement. Mixed hay and water were available ad libitum. Pens were cleaned regularly according to farm management protocols. Daily dry matter intake is shown in Table 2 .

Table 2 Dry matter intake of sheep

Items	Control group	Trial group I	Trial group II
Concentrate supplement			
Corn silage			
Alfalfa hay			
Wheat straw			
Total			

1.5 Sample Collection and Processing Blood samples were collected on the first day after the feeding trial at the following time points: pre-feeding (07:30, 19:30, designated as 0 h), and at 2, 4, 6, 8, and 10 h after morning and afternoon feeding. Blood was collected via jugular venipuncture into heparinized tubes, centrifuged at 3,500 r/min for 15 min to prepare plasma. A portion of plasma was carefully transferred to 1.5 mL Eppendorf tubes and stored at -20°C. Another portion was transferred to Amicon® Ultra-4 ultrafiltration tubes (Merck Millipore, molecular weight cutoff 10 kDa), centrifuged at 4,000 r/min for 20 min, and the filtrate was collected and stored at -20°C. During nighttime sampling, a dim flashlight covered with black cloth was used to minimize light exposure to the pineal gland.

1.6 Analytical Methods Plasma was analyzed for T-Trp, Kyn, ALB, FFA, 5-HT, and ML concentrations. Plasma collected at 0 h after morning feeding was also analyzed for T-AOC, GSH-Px activity, superoxide dismutase (SOD) activity, and MDA content. Ultrafiltrate was used for F-Trp determination. Plasma T-Trp and Kyn were measured by high-performance liquid chromatography. Plasma F-Trp was determined using the colorimetric method of Xie et al. Plasma ALB, FFA, T-AOC, GSH-Px, SOD, and MDA were analyzed by colorimetric assay at Nanjing Jiancheng Bioengineering Institute. Plasma 5-HT and ML were measured by enzyme-linked immunosorbent assay at Beijing Huaying Biotechnology Institute.

1.7 Statistical Analysis Data were organized using Excel 2010 and analyzed by one-way ANOVA using SPSS 19.0 software. Duncan's multiple range test was used for post-hoc comparisons. Results are expressed as mean±SD. Significance was set at $P<0.05$ and $P<0.01$.

Results

2.1 Plasma Total Tryptophan (T-Trp) Concentrations As shown in Table 3 , after morning feeding, plasma T-Trp in the control group decreased initially then increased from 0-6 h, remaining stable at 30.61-32.95 mol/L from 6-10 h. Trial group I increased rapidly from 0-2 h, becoming significantly higher than the control group at 2 h ($P<0.01$), then decreased sharply from 2-4 h, following a similar pattern to the control group from 4-10 h with no significant differences ($P>0.05$). Trial group II showed consistently higher T-Trp than the control group with a similar trend, being significantly higher at 6 h ($P<0.05$). Afternoon feeding produced similar trends. Overall, Trp supplementation caused a rapid short-term increase in plasma T-Trp, while RPTrp produced sustained modest elevations.

Table 3 Effects of Trp and RPTrp supplementation on plasma T-Trp content of sheep (n=5) mol/L

Sampling time	Clock time	Control group	Trial group I	Trial group II
0 h after morning feeding	07:30	34.20±5.06	32.95±4.95	33.29±3.86
2 h after morning feeding	10:00	32.33±4.51	42.01±5.02Aa	32.99±2.61
4 h after morning feeding	12:00	35.71±6.92	33.26±4.90ABb	34.68±7.43
6 h after morning feeding	14:00	28.74±2.77Bb	30.61±5.07	33.76±3.77
8 h after morning feeding	16:00	31.51±3.13	30.33±11.16	33.08±6.88
10 h after morning feeding	18:00	33.54±5.66	31.15±9.42	33.77±7.88
0 h after afternoon feeding	19:30	31.59±5.22b	27.39±1.55Bb	34.10±5.71
2 h after afternoon feeding	22:00	32.08±7.55b	38.77±7.94Aa	32.12±2.12
4 h after afternoon feeding	00:00	36.14±4.21a	30.89±4.30ABab	35.09±7.79
6 h after afternoon feeding	02:00	30.12±4.98	34.20±10.14	31.98±5.29
8 h after afternoon feeding	04:00	25.70±1.95	28.86±3.93	33.44±4.25
10 h after afternoon feeding	06:00	25.76±1.68	26.13±3.64	31.13±5.21

In the same row, values with the same or no letter superscripts indicate no significant difference ($P>0.05$), different lowercase letters indicate significant difference ($P<0.05$), and different uppercase letters indicate highly significant difference ($P<0.01$). The same applies below.

2.2 Plasma Free Tryptophan (F-Trp) Concentrations As shown in Table 4 , after morning feeding, plasma F-Trp in the control group remained stable at 7.39-7.98 mol/L from 0-8 h, then increased from 7.56 to 9.26 mol/L between 8-10 h and afternoon pre-feeding. Trial group I was significantly higher than both control and trial group II at 2 h (P<0.01), with no significant difference between control and trial group II (P>0.05). Trial group II increased gradually from 2-6 h, becoming significantly higher than both control and trial group I at 6 h (P<0.05). Afternoon feeding produced similar trends in trial groups, while control group F-Trp decreased then increased, being significantly lower than trial groups at 4 h (P<0.05) with no significant difference between trial groups (P>0.05).

Table 4 Effects of Trp and RPTrp supplementation on plasma F-Trp content of sheep (n=5) mol/L

Sampling time	Clock time	Control group	Trial group I	Trial group II
0 h after morning feeding	07:30	7.98±0.91	7.35±0.50b	6.78±0.48b
2 h after morning feeding	10:00	7.24±1.25	8.64±1.28	8.80±1.10a
4 h after morning feeding	12:00	8.34±1.18	8.67±0.84	7.21±1.37
6 h after morning feeding	14:00	7.44±1.10Bb	9.00±1.62	7.61±1.54
8 h after morning feeding	16:00	11.05±2.84Aa	8.89±0.68	7.99±1.38
10 h after morning feeding	18:00	8.00±0.52Bb	9.21±1.09a	8.19±1.05
0 h after afternoon feeding	19:30	7.96±1.14	8.77±0.67	7.62±1.01
2 h after afternoon feeding	22:00	7.39±1.47b	8.61±0.73	8.40±1.34
4 h after afternoon feeding	00:00	7.56±1.23	9.80±2.21	9.46±1.80a

Sampling time	Clock time	Control group	Trial group I	Trial group II
6 h after afternoon feeding	02:00	8.93±1.82	7.60±0.70Bb	8.87±0.99
8 h after afternoon feeding	04:00	9.26±1.92	12.25±1.31Aa	9.39±0.93
10 h after afternoon feeding	06:00	8.25±1.42	8.50±0.73Bb	9.11±1.22

2.3 Plasma Albumin (ALB) Concentrations As shown in Table 5 , after morning feeding, plasma ALB in the control group increased then decreased, while trial groups showed similar trends but with delayed peaks at 4 and 6 h for trial groups I and II, respectively. No significant differences were observed among groups (P>0.05). After afternoon feeding, all groups showed similar biphasic patterns with peaks at 4 and 10 h, again with no significant differences (P>0.05).

Table 5 Effects of Trp and RPTrp supplementation on plasma ALB content of sheep (n=5)

Sampling time	Clock time	Control group	Trial group I	Trial group II
0 h after morning feeding	07:30	27.83±4.02	28.05±1.37	26.74±2.87
2 h after morning feeding	10:00	24.64±5.90	28.00±5.31	23.88±0.94
4 h after morning feeding	12:00	25.36±2.66	30.18±2.96	23.00±1.90
6 h after morning feeding	14:00	32.65±8.43	25.48±6.84	28.86±3.93
8 h after morning feeding	16:00	27.09±2.94	26.23±0.98	26.39±3.41
10 h after morning feeding	18:00	29.48±5.51	28.17±7.73	24.06±3.16

Sampling time	Clock time	Control group	Trial group I	Trial group II
0 h after afternoon feeding	19:30	31.02±3.54	26.74±2.87	31.58±8.77
2 h after afternoon feeding	22:00	33.01±1.14	23.88±0.94	29.14±5.36
4 h after afternoon feeding	00:00	31.13±7.58	23.00±1.90	27.50±5.61
6 h after afternoon feeding	02:00	31.41±8.26	28.86±3.93	25.70±1.95
8 h after afternoon feeding	04:00	28.99±6.23	26.39±3.41	25.76±1.68
10 h after afternoon feeding	06:00	33.09±11.87	24.06±3.16	26.13±3.64

2.4 Plasma Free Fatty Acids (FFA) Concentrations As shown in Table 6 , after morning feeding, plasma FFA in all groups decreased then increased. Trial groups had lower values than the control from 4 h through afternoon pre-feeding, but differences were not significant ($P>0.05$). After afternoon feeding, similar trends were observed, but trial groups decreased more rapidly from 0-2 h, becoming significantly lower than the control at 2 h ($P<0.01$). No significant differences were found at other time points ($P>0.05$).

Table 6 Effects of Trp and RPTrp supplementation on plasma FFA content of sheep (n=5)

Sampling time	Clock time	Control group	Trial group I	Trial group II
0 h after morning feeding	07:30	164.47±42.73	105.30±18.93	177.36±29.91
2 h after morning feeding	10:00	175.16±47.38	91.82±8.98	163.02±15.00
4 h after morning feeding	12:00	156.48±42.50	95.28±23.34	163.10±19.95

Sampling time	Clock time	Control group	Trial group I	Trial group II
6 h after morning feeding	14:00	96.60±24.91	113.71±33.16	139.37±21.32
8 h after morning feeding	16:00	111.70±25.78	98.87±18.71	134.53±35.26
10 h after morning feeding	18:00	113.02±24.98	114.21±19.15	130.90±23.36
0 h after afternoon feeding	19:30	114.53±10.10	104.53±12.51	162.26±21.93Aa
2 h after afternoon feeding	22:00	162.26±21.93Aa	101.13±8.81Bb	110.69±14.46B
4 h after afternoon feeding	00:00	114.78±19.78	120.34±24.51	100.63±11.12
6 h after afternoon feeding	02:00	123.52±28.38	112.96±46.25	96.35±25.02
8 h after afternoon feeding	04:00	130.06±15.83	117.74±25.28	182.14±38.74
10 h after afternoon feeding	06:00	136.10±46.44	165.53±44.74	179.87±64.07

2.5 Plasma Kynurenine (Kyn) Concentrations As shown in Table 7 , after morning feeding, plasma Kyn in the control and trial group II remained relatively stable at 3.19-3.79 mol/L and 3.56-4.35 mol/L, respectively, with trial group II consistently higher but not significantly different (P>0.05). Trial group I increased rapidly from 0-2 h, becoming significantly higher than both other groups at 2 h (P<0.05). After afternoon feeding, control and trial group II increased gradually from 2-8 h with no significant difference (P>0.05), while trial group I showed a similar pattern to the morning, being significantly higher at 2 h (P<0.05).

Table 7 Effects of Trp and RPTrp supplementation on plasma Kyn content of sheep (n=5) mol/L

Sampling time	Clock time	Control group	Trial group I	Trial group II
0 h after morning feeding	07:30	3.79±0.37	3.65±0.11	3.77±0.49
2 h after morning feeding	10:00	3.27±0.63b	4.05±0.39	4.01±0.73
4 h after morning feeding	12:00	3.26±0.38	4.35±0.87	4.06±0.22
6 h after morning feeding	14:00	3.27±0.09	3.74±0.55b	4.17±0.44
8 h after morning feeding	16:00	3.19±0.33	4.11±1.40	4.71±0.78
10 h after morning feeding	18:00	3.56±0.65	3.56±0.78	5.06±0.43
0 h after afternoon feeding	19:30	4.10±0.55	3.77±0.49	4.62±0.49
2 h after afternoon feeding	22:00	3.67±0.02	5.54±1.40a	4.06±0.33
4 h after afternoon feeding	00:00	5.54±1.40a	4.35±0.66	4.35±0.17
6 h after afternoon feeding	02:00	4.35±0.66	3.55±0.34	3.96±1.05
8 h after afternoon feeding	04:00	3.55±0.34	4.61±0.33	4.18±0.56
10 h after afternoon feeding	06:00	3.34±0.30	5.55±1.41a	4.82±0.75

2.6 Plasma 5-Hydroxytryptamine (5-HT) Concentrations As shown in Table 8 , after morning feeding, plasma 5-HT in all groups decreased initially, then increased, then decreased again, peaking at 8 h. At 10 h, trial group II was significantly higher than the control group ($P<0.05$), with no significant difference between trial group I and other groups ($P>0.05$). After afternoon

feeding, 5-HT increased gradually from 0-6 h, then more rapidly from 6-10 h. At 8 h, trial group II was significantly higher than both control and trial group I ($P<0.05$), while the control and trial group I did not differ significantly ($P>0.05$).

Table 8 Effects of Trp and RPTrp supplementation on plasma 5-HT content of sheep (n=5) ng/mL

Sampling time	Clock time	Control group	Trial group I	Trial group II
0 h after morning feeding	07:30	374.36±88.06	302.51±74.23	285.39±44.10b
2 h after morning feeding	10:00	383.59±49.41	334.04±57.65	251.38±67.57b
4 h after morning feeding	12:00	366.51±82.14	308.37±68.14	376.59±69.99a
6 h after morning feeding	14:00	242.46±30.97	164.72±23.29b	338.73±96.03
8 h after morning feeding	16:00	278.23±57.97	185.86±53.28ab	377.21±74.27
10 h after morning feeding	18:00	288.91±48.60	234.73±51.44a	417.02±45.31
0 h after afternoon feeding	19:30	209.63±59.48	154.03±37.63	338.73±96.03
2 h after afternoon feeding	22:00	200.17±26.36	169.61±44.52	377.21±74.27
4 h after afternoon feeding	00:00	240.34±58.62	171.50±43.50	417.02±45.31
6 h after afternoon feeding	02:00	243.86±39.68	194.09±54.12	189.73±30.40
8 h after afternoon feeding	04:00	238.13±58.74	186.12±34.32	246.00±34.88

10 h after afternoon feeding	06:00	231.96±70.24	197.35±34.63	197.92±51.60

2.7 Plasma Melatonin (ML) Concentrations As shown in Table 9 , after morning feeding, plasma ML in trial groups increased continuously from 2-8 h, being significantly higher than the control group at 6 and 8 h (P<0.01), with no significant difference between trial groups (P>0.05). The control group showed minimal increase. After afternoon feeding, trial group II maintained the smallest variation (87.19-98.34 pg/mL) and was consistently higher than other groups, being significantly higher than the control at 4 and 8 h (P<0.05). No significant difference was observed between trial group I and the control (P>0.05).

Table 9 Effects of Trp and RPTrp supplementation on plasma ML content of sheep (n=5) pg/mL

Sampling time	Clock time	Control group	Trial group I	Trial group II
0 h after morning feeding	07:30	72.42±10.58	65.52±12.07Bb	54.47±10.99b
2 h after morning feeding	10:00	67.15±12.42	91.04±15.46Aa	74.78±11.62ab
4 h after morning feeding	12:00	83.93±11.80	108.89±12.20Aa	90.80±16.03a
6 h after morning feeding	14:00	71.70±17.25	84.40±20.03Bb	76.69±11.85
8 h after morning feeding	16:00	54.85±12.17	134.11±19.55Aa	63.85±12.08
10 h after morning feeding	18:00	57.60±14.68	140.34±39.11Aa	87.19±20.46
0 h after afternoon feeding	19:30	58.59±10.29	87.66±15.84	53.90±10.63b
2 h after afternoon feeding	22:00	76.37±16.00	76.68±12.91	70.27±12.16b
4 h after afternoon feeding	00:00	72.02±12.30	103.18±23.96	95.34±17.90a

Sampling time	Clock time	Control group	Trial group I	Trial group II
6 h after afternoon feeding	02:00	87.37±13.06	92.97±15.11	85.86±9.97
8 h after afternoon feeding	04:00	92.97±15.11	108.51±11.41	84.36±21.77
10 h after afternoon feeding	06:00	108.51±11.41	93.04±14.68	94.03±20.84

2.8 Plasma Antioxidant Capacity As shown in Table 10 , trial groups exhibited significantly higher plasma GSH-Px activity and lower MDA content compared to the control group (P<0.01). Trial group II also showed significantly higher plasma T-AOC than both control and trial group I (P<0.01).

Table 10 Effects of Trp and RPTrp supplementation on plasma antioxidant capacity of sheep (n=5)

Items	Control group	Trial group I	Trial group II
Total antioxidant capacity T-AOC (U/mL)	1.48±0.12Bb	1.32±0.07Bb	2.47±0.32Aa
Glutathione peroxidase GSH-Px (U/mL)	21.05±1.70Bb	28.36±2.69Aa	31.00±1.98Aa
Superoxide dismutase SOD (U/mL)	80.73±1.73	78.46±1.13	81.03±1.18
Malondialdehyde MDA (nmol/mL)	3.80±0.83Aa	2.10±0.30Bb	2.07±0.18Bb

Discussion

3.1 Effects on Plasma T-Trp, F-Trp, FFA, and ALB This study demonstrated that after morning and afternoon supplementation with Trp or RPTrp, plasma T-Trp and F-Trp in trial group I were significantly elevated at 2 h (10:00, 22:00) compared to the control group. Research indicates that after intraruminal administration of radiolabeled [^{1} C] Trp, Trp can be detected in portal plasma within 10 min, with 25-70% absorbed within 3 h. The rapid increase in plasma T-Trp and F-Trp in trial group I from 0-2 h may be attributed to rapid rumen bypass of soluble Trp or the method of mixing Trp with 50 g concentrate before feeding. Trial group II showed significantly higher T-Trp and F-Trp at 2 h and 6 h (14:00). Kollmann et al. reported that supplementing dairy cows with 500 g/d RPTrp (25% Trp) significantly increased plasma T-Trp during both day and night, and supplementing cashmere goats with 6 g/d RPTrp (33% Trp) significantly elevated plasma T-Trp, consistent with our findings. The decrease in trial group I T-Trp from 2-4 h (10:00-12:00, 22:00-24:00) below control levels, while trial group II maintained higher levels, indicates that RPTrp effectively prevented ruminal degradation. The lack of significant difference in trial group II T-Trp after afternoon feeding, inconsistent with Kollmann et al., may be related to feeding time and diet composition.

Plasma FFA in all groups showed similar diurnal patterns of decrease followed by increase, reflecting that FFA absorption primarily occurs in the distal jejunum with slower ruminal absorption. Nicotinic acid, a major Trp metabolite, exhibits antilipolytic effects. Dairy cows fed 12 g/d nicotinic acid showed significantly reduced plasma FFA. The decreased plasma FFA in trial groups may be associated with increased plasma nicotinic acid. Plasma ALB is metabolically stable, contains minimal Trp residues, has a long half-life (12.7-18.2 d), and is regulated primarily by colloid osmotic pressure. The absence of significant differences in plasma ALB among groups indicates that Trp or RPTrp supplementation does not significantly affect ALB concentrations.

3.2 Effects on Plasma Kyn Plasma Kyn showed similar temporal patterns to T-Trp and F-Trp following supplementation, consistent with studies in healthy adult men showing increased plasma Kyn after oral Trp loading. Approximately 95% of Trp is metabolized via the kynurenine pathway in mammals, primarily catalyzed by hepatic tryptophan-2,3-dioxygenase (TDO). Rats injected with 100 mg/(kg BW · d) Trp showed increased plasma F-Trp and hepatic TDO activity at 3 h. Additionally, dietary Trp supplementation increased hepatic TDO content in piglets. Our results likely reflect increased hepatic TDO activity or content in sheep.

3.3 Effects on Plasma 5-HT Plasma 5-HT in trial group II was significantly higher than the control group at 10 h after morning feeding (16:00) and 8 h after afternoon feeding (04:00), while trial group I showed no significant differences from the control at any time. 5-HT is a major Trp metabolite produced

primarily by intestinal enterochromaffin cells (EC), with smaller contributions from mast cells, pancreatic -cells, adipocytes, and osteoblasts. Tryptophan hydroxylase (TPH) is the rate-limiting enzyme. Studies show that increasing dietary Trp from 0.11% to 0.24% significantly elevated serum 5-HT in piglets, consistent with our findings. However, RPTrp supplementation did not significantly increase plasma 5-HT in lactating dairy cows, possibly due to lower TPH activity or content in EC cells. TPH has a Km of approximately 50 mol/L and its activity is influenced by substrate Trp and cofactor tetrahydrobiopterin availability; insufficient TPH or cofactor may limit 5-HT synthesis.

3.4 Effects on Plasma ML Plasma ML in trial groups was significantly higher than the control at 6-8 h after morning feeding (14:00-16:00). After afternoon feeding, trial group II maintained higher ML than both control and trial group I. Mammalian plasma ML originates primarily from intestinal EC during daytime and pineal gland at night, showing clear circadian rhythms. Supplementing non-pregnant Brown Swiss heifers with 500 g/d RPTrp (25% Trp) significantly increased daytime and nighttime plasma ML, consistent with our results. Studies in pigs show peak plasma ML at 5 h post-feeding, correlating with ML content in the ileum, cecum, and colon. The increase in trial group ML from 4-8 h after morning feeding (12:00-16:00) may reflect elevated intestinal ML synthesis. Future studies should examine the relationship between plasma and intestinal ML concentrations.

Peak plasma ML in trial groups occurred at 8 h after morning feeding (16:00), while the control group peaked at 2 h after afternoon feeding (22:00), without clear circadian patterns. This may be explained by: (1) seasonal variation in plasma ML—under long-day conditions (15.27 h photoperiod), pineal N-acetyltransferase expression decreases, reducing nocturnal pineal ML secretion; (2) significant individual variation in sheep plasma ML; and (3) bilateral jugular vein differences in nocturnal ML concentrations.

3.5 Effects on Plasma Antioxidant Capacity Trial groups showed significantly higher plasma GSH-Px activity and lower MDA content compared to the control group ($P<0.01$), with trial group II also exhibiting significantly higher T-AOC. Studies demonstrate that dietary Trp supplementation in weaned piglets significantly increases serum T-AOC and decreases MDA. Supplementing dairy cows with 220 g/d RPTrp (45% Trp) significantly elevated plasma GSH-Px activity. These effects likely result from increased plasma Trp and ML concentrations. The amino group in Trp can bind oxidants, preventing oxidative reactions and reducing MDA. ML exerts direct antioxidant effects and enhances antioxidant enzyme activity; its metabolites N-acetyl-N-formyl-5-methoxykynuramine and 6-hydroxymelatonin possess even stronger antioxidant properties.

Conclusion

1. Trp supplementation rapidly increased plasma T-Trp and F-Trp concentrations post-feeding and accelerated Trp metabolism via the kynurenine pathway, while RPTrp produced more moderate effects.
2. Trp and RPTrp supplementation had minimal effects on plasma ALB, FFA, and 5-HT concentrations, with significant differences only at isolated time points.
3. Supplementation increased daytime plasma ML concentrations. Trp had no significant effect on nighttime ML, while RPTrp maintained nighttime plasma ML at relatively elevated levels.
4. Both Trp and RPTrp supplementation enhanced plasma antioxidant capacity in sheep.

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