Effects of Dietary Supplementation with 5-Hydroxytryptophan and Rumen-Protected 5-Hydroxytryptophan on Plasma 5-Hydroxytryptophan and Melatonin Concentrations in Sheep (Post-print)

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Abstract

This study aimed to investigate the effects of dietary supplementation with 5-hydroxytryptophan and rumen-protected 5-hydroxytryptophan on plasma 5-hydroxytryptophan and melatonin concentrations in sheep, and to explore the possibility of regulating melatonin secretion patterns and concentrations through 5-hydroxytryptophan. Eighteen 1-year-old Dorset ewes with an average body weight of (55.78±3.24) kg were selected and divided into three groups based on body weight (n=6 per group): a control group, experimental group I, and experimental group II. The daily ration consisted of powdered concentrate at 1% of body weight, corn silage at 0.6 kg per head, and mixed hay provided ad libitum. Additionally, sheep in experimental groups I and II were supplemented with 5-hydroxytryptophan at 50 mg/kg BW and rumen-protected 5-hydroxytryptophan at 111 mg/kg BW, respectively, for a 15-day feeding trial. The results showed that plasma 5-hydroxytryptophan concentrations in the experimental groups were higher than those in the control group after both morning and afternoon feeding, exhibiting fluctuating elevations after afternoon feeding, though the difference between experimental groups I and II was not significant ($P > 0.05$). Plasma tryptophan concentrations in all groups showed an increasing trend at 1.5 h after morning feeding, while decreasing at 1.5–6.0 h after afternoon feeding. During daytime, no significant differences in plasma 5-hydroxytryptamine concentrations were observed among groups ($P > 0.05$); however, concentrations showed fluctuating increases at 1.5–6.0 h after afternoon feeding. At 3.0–9.0 h after morning feeding, plasma melatonin concentrations in experimental groups I and II were higher than in the control group, with differences being highly significant at 6.0 h after

morning feeding ($P < 0.01$). At 1.5–9.0 h after afternoon feeding, plasma melatonin concentrations in all groups exhibited fluctuating increases, with experimental groups I and II being highly significantly higher than the control group at 1.5, 3.0, and 6.0 h ($P < 0.01$), and experimental group II being significantly higher than the control group at 9.0 h ($P < 0.05$). It can be concluded that dietary supplementation with either 5-hydroxytryptophan (50 mg/kg BW) or rumen-protected 5-hydroxytryptophan (111 mg/kg BW) can significantly increase plasma 5-hydroxytryptophan concentrations and alter daytime melatonin secretion patterns and concentrations in sheep, with rumen-protected 5-hydroxytryptophan being more effective, but has no significant effect on plasma tryptophan or 5-hydroxytryptamine concentrations.

Full Text

Effects of Diet Supplemented with 5-Hydroxytryptophan, Rumen Protected 5-Hydroxytryptophan on Plasma 5-Hydroxytryptophan and Melatonin Contents of Sheep

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Abstract

This study investigated the effects of dietary supplementation with 5-hydroxytryptophan (5-HTP) and rumen-protected 5-hydroxytryptophan on plasma concentrations of 5-HTP and melatonin in sheep, exploring the potential for regulating melatonin secretion patterns and concentrations through 5-HTP administration. Eighteen healthy one-year-old Dorset ewes with an average body weight of (55.78±3.24) kg were randomly allocated into three groups (n=6 per group): a control group, Trial Group I, and Trial Group II. All sheep received a powdered concentrate at 1% of body weight, 0.6 kg of corn silage daily, and ad libitum access to mixed hay. Trial Group I received supplemental 5-HTP at 50 mg/kg BW, while Trial Group II received rumen-protected 5-HTP at 111 mg/kg BW (providing an equivalent effective 5-HTP dose of 50 mg/kg BW). The 15-day feeding trial revealed that plasma 5-HTP concentrations in both trial groups were elevated compared to the control group following morning and afternoon feedings, showing a fluctuating increase after afternoon feeding, though differences between the two trial groups were not significant ($P>0.05$). Plasma tryptophan concentrations increased at 1.5 hours post-feeding in the morning across all groups, but declined between 1.5 and 6.0 hours after afternoon feeding. During daytime hours, no significant differences in plasma 5-hydroxytryptamine (5-HT) concentrations were observed among

groups (P>0.05), though levels showed a fluctuating increase from 1.5 to 6.0 hours after afternoon feeding. Plasma melatonin concentrations in both trial groups exceeded those of the control group between 3.0 and 9.0 hours after morning feeding, with highly significant differences at 6.0 hours post-feeding (P<0.01). Following afternoon feeding, melatonin concentrations in all groups exhibited a fluctuating upward trend from 1.5 to 9.0 hours, with both trial groups showing highly significant elevations compared to the control group at 1.5, 3.0, and 6.0 hours (P<0.01), and Trial Group II maintaining significantly higher levels at 9.0 hours (P<0.05). These findings demonstrate that dietary supplementation with either 5-HTP (50 mg/kg BW) or rumen-protected 5-HTP (111 mg/kg BW) significantly increases plasma 5-HTP concentrations and modulates daytime melatonin secretion patterns and concentrations in sheep, with rumen-protected 5-HTP demonstrating superior efficacy, while neither treatment significantly affects plasma tryptophan or 5-HT concentrations.

Keywords: sheep; melatonin; 5-hydroxytryptophan; 5-hydroxytryptamine

Introduction

Melatonin is widely distributed across unicellular algae, plants, invertebrates, and higher animals. While the pineal gland represents a primary site of melatonin synthesis in animals, intestinal melatonin concentrations are approximately 400-fold higher than those in the pineal gland [1]. Melatonin exerts diverse biological functions, including acting as a non-enzymatic antioxidant that scavenges hydroxyl radicals and various reactive oxygen species while enhancing antioxidant enzyme expression and activity [2]. Additionally, melatonin regulates reproductive function, modulates follicular development and oocyte maturation, and promotes embryonic development [3], while exerting stimulatory effects on both innate and acquired immunity [4]. Given these broad biological roles, enhancing melatonin concentrations during specific physiological stages may yield beneficial outcomes.

Current approaches to modulating endogenous melatonin levels include direct intravenous injection or dietary melatonin supplementation [5-7], and administration of melatonin precursors such as tryptophan (Try) and 5-hydroxytryptophan (5-HTP) [1,8]. However, studies in ruminants have shown that dietary tryptophan or rumen-protected tryptophan supplementation does not significantly affect plasma melatonin concentrations in lactating cows during day or night [9]. While intraperitoneal injection of 500 mg/kg BW tryptophan failed to increase plasma melatonin in sheep, 5-HTP administration significantly elevated plasma melatonin concentrations [8], suggesting species-specific differences in tryptophan efficacy as a melatonin precursor. Although intraperitoneal 5-HTP affects plasma melatonin in sheep, the efficacy of oral administration remains unclear. Furthermore, given the extensive degradation of amino acids by rumen microorganisms and the absence of research on 5-HTP degradation in the ovine rumen, this study compared plasma concentrations of 5-HTP, 5-hydroxytryptamine (5-HT), and melatonin at various time points following supplementation with 5-

HTP or rumen-protected 5-HTP. The objective was to explore the potential for regulating melatonin secretion patterns and concentrations through dietary 5-HTP supplementation, providing a theoretical foundation for manipulating melatonin synthesis in ruminants via feed additives.

Materials and Methods

1.1 Experimental Period and Location

The experiment was conducted from May 13 to May 29, 2017, at the Xinjiang Huikang Animal Husbandry Biotechnology Co., Ltd. sheep farm. Sunrise occurred at 06:32 and sunset at 21:44 on blood sampling days.

1.2 Experimental Materials

5-HTP (98% purity) was purchased from Wuhan Yuancheng Gongchuang Technology Co., Ltd. Rumen-protected 5-HTP was manufactured by Beijing Yahe Nutrition High-tech Co., Ltd., containing 45.00% effective 5-HTP with a rumen bypass rate of 88.60%.

1.3 Experimental Design

Eighteen healthy one-year-old Dorset ewes with an average body weight of (55.78±3.24) kg (range: 50.5–65.8 kg) were randomly divided into three groups (n=6 per group) based on body weight: control, Trial Group I, and Trial Group II. All sheep received a powdered concentrate (purchased from Xinjiang Tiankang Animal Husbandry Biotechnology Co., Ltd.) at 1% of body weight daily, 0.6 kg corn silage, and ad libitum mixed hay (alfalfa:wheat straw = 3:7). Trial Group I received supplemental 5-HTP at 50 mg/kg BW, while Trial Group II received rumen-protected 5-HTP at 111 mg/kg BW, providing equivalent effective 5-HTP doses of 50 mg/kg BW based on Huether et al. [10]. The 15-day feeding trial utilized dietary compositions and nutrient levels shown in Table 1 and Table 2 .

1.4 Feeding Management

During the trial, daily rations of powdered concentrate, corn silage, and 5-HTP supplements were divided equally for feeding at 07:30 (morning) and 19:30 (afternoon). The 5-HTP or rumen-protected 5-HTP was mixed with 50 g of powdered concentrate and fed first; remaining concentrate and corn silage were offered after complete consumption. Sheep were individually penned during feeding and allowed free movement in exercise areas afterward. Nighttime housing provided 250 lx illumination.

1.5 Sample Collection and Processing

Blood samples were collected on day 16 at 0 hours before morning feeding (07:30) and afternoon feeding (19:30), and at 1.5, 3.0, 6.0, and 9.0 hours post-feeding.

Jugular venipuncture into heparinized tubes was followed by centrifugation at 3,500 r/min for 15 minutes to harvest plasma, which was immediately transferred to 1.5 mL Eppendorf tubes and stored at -20°C. Blood collection at each time point was completed within 10 minutes per animal.

1.6 Sample Analysis

Plasma samples were analyzed at Beijing Huaying Biotechnology Research Institute for 5-HTP, tryptophan, 5-HT, and melatonin concentrations. Radioimmunoassay (XH-6020 automatic radioimmunoassay counter, Xi' an Nuclear Instrument Factory) was used for 5-HTP and melatonin, enzyme-linked immunosorbent assay (Huaweidelang DR-200BS microplate reader, Wuxi Huaweidelang Instrument Co., Ltd.) for 5-HT, and colorimetric methods (A6 semi-automatic biochemical analyzer, Beijing Songshang Technology Co., Ltd.) for tryptophan.

1.7 Statistical Analysis

Data were initially processed using Excel 2003 and expressed as mean ± standard deviation (SD). One-way ANOVA was performed using SPSS 18.0 software, with Duncan' s multiple range test for intergroup comparisons.

Results

2.1 Effects of 5-HTP and Rumen-Protected 5-HTP on Plasma 5-HTP Concentrations

As shown in Table 3 and Figure 1 [Figure 1: see original paper], plasma 5-HTP concentrations in both trial groups exceeded those of the control group at 1.5, 3.0, 6.0, and 9.0 hours after morning feeding (09:00–17:00). Trial Group I showed highly significant differences from the control group at 1.5, 3.0, and 9.0 hours ($P<0.01$), while Trial Group II differed significantly at 1.5 and 9.0 hours ($P<0.01$). At 0 hours before afternoon feeding (19:30), 5-HTP concentrations were similar across groups ($P>0.05$). Following afternoon feeding, both trial groups maintained higher 5-HTP concentrations than the control group, with highly significant differences at 1.5 hours (21:00) ($P<0.01$). Trial groups exhibited increasing 5-HTP concentrations from 1.5 to 6.0 hours post-feeding, whereas the control group showed a decline at 1.5 hours.

Table 3 Effects of dietary 5-HTP and rumen-protected 5-HTP supplementation on plasma 5-HTP concentrations in sheep (n=6) (g/L)

Sampling Time Points	Clock Time	Control Group	Trial Group I	Trial Group II
0 h before morning feeding	07:30	249.43±30.85	278.66±33.30	294.54±52.40

Sampling Time Points	Clock Time	Control Group	Trial Group I	Trial Group II
1.5 h after morning feeding	09:00	185.76±20.79Bb	238.92±29.80Aa	275.89±51.81Aa
3.0 h after morning feeding	11:00	199.39±16.89Bb	325.11±33.39Aa	223.23±19.05Bb
6.0 h after morning feeding	14:00	217.68±23.09	246.31±24.36	237.31±26.94
9.0 h after morning feeding	17:00	217.76±29.22	219.76±21.66	261.51±36.96
0 h before afternoon feeding	19:30	177.83±21.77Bb	260.16±18.35Aa	243.13±29.30Aa
1.5 h after afternoon feeding	21:00	212.37±21.96Bb	270.58±29.90Aa	277.79±47.54Aa
3.0 h after afternoon feeding	23:00	265.12±46.32	284.73±53.57	270.60±27.97
6.0 h after afternoon feeding	02:00	249.12±23.84	295.80±45.86	290.48±30.51
9.0 h after afternoon feeding	05:00	212.47±15.96	265.12±46.32	284.73±53.57

Note: In the same row, values with no letter or the same 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.

Figure 1 Effects of dietary supplementation with 5-HTP and rumen-protected 5-HTP on plasma 5-HTP concentrations in sheep. “ ” indicates feeding times, and “ ” indicates nighttime hours. The same applies below.

2.2 Effects of 5-HTP and Rumen-Protected 5-HTP on Plasma Tryptophan Concentrations

As presented in Table 4 and Figure 2 [Figure 2: see original paper], plasma tryptophan concentrations increased across all groups at 1.5 hours after morning feeding (09:00). From 3.0 to 6.0 hours post-feeding, the control and Trial Group II showed initial declines followed by increases, whereas Trial Group I demonstrated continuous elevation, though without significant intergroup differences at any sampling point (P>0.05). Tryptophan concentrations peaked at 0 hours before afternoon feeding (19:30) in all groups, with the control group showing higher values than trial groups (P>0.05). Following afternoon feeding, tryptophan concentrations declined from 1.5 to 6.0 hours (21:00–02:00) in all groups, with no significant differences between groups at identical time points. At 9.0 hours after afternoon feeding (05:00), both trial groups exhibited increasing tryptophan concentrations while the control group continued to decline.

Table 4 Effects of dietary 5-HTP and rumen-protected 5-HTP supplementation on plasma tryptophan concentrations in sheep (n=6) (mol/L)

Sampling Time Points	Clock Time	Control Group	Trial Group I	Trial Group II
0 h before morning feeding	07:30	34.13±3.96	33.05±1.63	33.83±3.49
1.5 h after morning feeding	09:00	32.24±2.25	36.28±4.82	37.64±2.00
3.0 h after morning feeding	11:00	32.72±2.90	32.99±3.14	37.58±4.62
6.0 h after morning feeding	14:00	35.20±2.20	35.15±2.42	37.13±3.30
9.0 h after morning feeding	17:00	37.38±1.29	41.23±5.88	41.55±7.12
0 h before afternoon feeding	19:30	33.87±1.93	41.85±4.29	38.62±2.33
1.5 h after afternoon feeding	21:00	34.90±4.20	36.33±2.73	35.84±2.13
3.0 h after afternoon feeding	23:00	45.03±8.94	38.21±3.99	35.17±3.91
6.0 h after afternoon feeding	02:00	41.23±5.88	37.90±3.39	32.83±1.41
9.0 h after afternoon feeding	05:00	41.85±4.29	36.37±5.04	34.89±4.76

Figure 2 Effects of dietary supplementation with 5-HTP and rumen-protected 5-HTP on plasma tryptophan concentrations in sheep.

2.3 Effects of 5-HTP and Rumen-Protected 5-HTP on Plasma 5-HT Concentrations

As illustrated in Table 5 and Figure 3 [Figure 3: see original paper], plasma 5-HT concentrations ranged from 320–285 ng/mL across all groups at 0, 1.5, 3.0, 6.0, and 9.0 hours after morning feeding (07:30–17:00), with no significant intergroup differences ($P>0.05$). At 0 hours before afternoon feeding (19:30), all groups exhibited decreased 5-HT concentrations, with trial groups showing lower values than the control group ($P>0.05$). Subsequently, 5-HT concentrations showed a fluctuating increase from 1.5 to 6.0 hours after afternoon feeding (21:00–02:00), followed by a decline at 9.0 hours (05:00) across all groups.

Table 5 Effects of dietary 5-HTP and rumen-protected 5-HTP supplementation on plasma 5-HT concentrations in sheep (n=6) (ng/mL)

Sampling Time Points	Clock Time	Control Group	Trial Group I	Trial Group II
0 h before morning feeding	07:30	315.57±71.28	316.88±48.05	340.72±15.52
1.5 h after morning feeding	09:00	320.44±62.30	331.23±43.47	318.57±37.23
3.0 h after morning feeding	11:00	302.11±54.82	320.78±49.70	292.79±51.58
6.0 h after morning feeding	14:00	303.69±41.53	305.10±45.33	298.08±27.51
9.0 h after morning feeding	17:00	318.41±34.64	320.50±48.10	289.32±43.79
0 h before afternoon feeding	19:30	285.03±61.92	298.30±9.31	281.53±48.27
1.5 h after afternoon feeding	21:00	321.52±17.70	279.88±28.31	316.14±20.30
3.0 h after afternoon feeding	23:00	292.53±54.55	273.05±43.72	299.29±53.89
6.0 h after afternoon feeding	02:00	322.71±41.98	252.85±55.91	282.51±80.29
9.0 h after afternoon feeding	05:00	312.38±54.09	286.90±67.46	282.40±58.71

Figure 3 Effects of dietary supplementation with 5-HTP and rumen-protected 5-HTP on plasma 5-HT concentrations in sheep.

2.4 Effects of 5-HTP and Rumen-Protected 5-HTP on Plasma Melatonin Concentrations

As depicted in Table 6 and Figure 4 [Figure 4: see original paper], plasma melatonin concentrations in both trial groups were significantly lower than the control group at 1.5 hours after morning feeding (09:00) ($P<0.01$). From 3.0 to 9.0 hours post-feeding (11:00–17:00), trial groups exhibited higher melatonin concentrations than the control group, with highly significant differences at 6.0 hours (14:00) ($P<0.01$). After afternoon feeding, all groups showed fluctuating increases in melatonin concentrations from 1.5 to 9.0 hours, with trial groups displaying highly significant elevations compared to the control group at 1.5, 3.0, and 6.0 hours ($P<0.01$). Trial Group II maintained significantly higher concentrations than the control group at 9.0 hours ($P<0.05$).

Table 6 Effects of dietary 5-HTP and rumen-protected 5-HTP supplementation on plasma melatonin concentrations in sheep (n=6) (pg/mL)

Sampling Time Points	Clock Time	Control Group	Trial Group I	Trial Group II
0 h before morning feeding	07:30	59.15±3.91	52.15±4.50	45.94±5.72
1.5 h after morning feeding	09:00	51.89±6.87	41.34±8.51Bb	36.40±7.90Bb
3.0 h after morning feeding	11:00	45.87±8.16	75.73±10.98Aa	82.31±10.68Aa
6.0 h after morning feeding	14:00	59.38±3.35Aa	91.41±20.92Aa	89.86±7.56Aa
9.0 h after morning feeding	17:00	38.39±5.24Bb	45.39±4.93Bb	37.26±8.47
0 h before afternoon feeding	19:30	53.19±5.00	38.07±8.32Bc	33.67±2.69Bb
1.5 h after afternoon feeding	21:00	53.98±7.20	69.16±18.91Ab	82.95±16.20Aa
3.0 h after afternoon feeding	23:00	41.34±8.51Bb	92.72±7.46Aa	89.86±7.56Aa
6.0 h after afternoon feeding	02:00	75.73±10.98Aa	55.18±5.43b	76.26±19.49ab
9.0 h after afternoon feeding	05:00	91.41±20.92Aa	76.26±19.49ab	96.77±20.79a

Figure 4 Effects of dietary supplementation with 5-HTP and rumen-protected 5-HTP on plasma melatonin concentrations in sheep.

Discussion

3.1 Effects on Plasma 5-HTP Concentrations

Dynamic changes in plasma 5-HTP concentrations in sheep around feeding have been rarely reported. The present study revealed that during daytime hours, control sheep exhibited a "W-shaped" pattern of plasma 5-HTP over 12 hours, with peaks occurring at 0 hours pre-feeding and 6.0 hours post-feeding. In Trial Group I (5-HTP supplementation), daytime plasma 5-HTP showed a "bimodal" pattern with peaks at 3.0 and 9.0 hours after morning feeding (11:00 and 17:00), resembling 5-HTP metabolism patterns in healthy humans and potentially related to gastric emptying phases and enterohepatic circulation [11]. Trial Group II (rumen-protected 5-HTP) displayed a "unimodal" pattern, peaking at 6.0 hours post-feeding.

From afternoon feeding through nighttime (19:30–05:00), all groups exhibited elevated 5-HTP concentrations compared to daytime averages. In control sheep, this likely reflected increased endogenous synthesis from intestinal and pineal sources, while trial groups benefited from both endogenous production and

supplemental 5-HTP. Human studies demonstrate that 70% of orally administered 5-HTP (2 mg/kg BW) enters systemic circulation within 2 hours [12]. In panic disorder patients, intravenous 5-HTP injection produced dose-dependent plasma elevations peaking at 30 minutes post-injection [13]. In the current study, 5-HTP concentrations were higher in Trial Group I than Trial Group II at 3.0 and 6.0 hours after morning feeding and 1.5 hours after afternoon feeding, likely due to rapid absorption of unprotected 5-HTP versus delayed release from rumen-protected formulations. Whether 5-HTP undergoes ruminal degradation requires further investigation.

3.2 Effects on Plasma Tryptophan Concentrations

Plasma tryptophan concentrations remained comparable between trial and control groups across all sampling times, with similar fluctuation patterns, indicating that 5-HTP supplementation did not significantly affect tryptophan absorption or metabolism. 5-HTP absorption occurs via active transport [14], while amino acids utilize specific transporters [15], suggesting non-competitive intestinal absorption mechanisms. The detailed mechanisms of 5-HTP intestinal transport remain unclear.

Plasma amino acid concentrations are influenced by feeding time and dietary composition [16]. Purser et al. [17] reported increased plasma amino acid concentrations 3 hours post-feeding in lambs consuming 8.1% crude protein diets. The current study' s observation of fluctuating increases in plasma tryptophan from 1.5 to 9.0 hours after morning feeding (09:00–19:30) aligns with these findings. The declining tryptophan concentrations from 1.5 to 6.0 hours after afternoon feeding (21:00–02:00) coincided with rising 5-HTP concentrations, suggesting conversion of tryptophan to 5-HTP via tryptophan hydroxylase. At 9.0 hours after afternoon feeding (05:00), trial groups showed increasing tryptophan concentrations while the control group continued declining. This period represents peak melatonin synthesis, during which supplemental 5-HTP may serve as an alternative precursor, reducing tryptophan consumption and allowing plasma accumulation. In contrast, control sheep relied solely on tryptophan for melatonin synthesis, leading to sustained tryptophan depletion.

3.3 Effects on Plasma 5-HT Concentrations

Plasma 5-HT concentrations remained similar between trial and control groups throughout the sampling period, with consistent fluctuation patterns. As both a conversion product of tryptophan/5-HTP and direct substrate for melatonin synthesis, 5-HT is primarily eliminated through urinary excretion. Mammalian liver and kidneys contain abundant 5-HT-degrading enzymes [18]; approximately two-thirds of 5-HT is conjugated with sulfate or glucuronic acid in the liver before excretion, while one-third is oxidatively deaminated by monoamine oxidase to 5-hydroxyindoleacetic acid [19]. Thus, blood 5-HT concentrations reflect a balance among synthesis, conversion, and metabolic elimination. Wa et al. [20] demonstrated that continuous intravenous 5-HTP

infusion (10 g/kg/min) for 60 minutes during daytime increased urinary 5-HT excretion from <0.7 to (412±92) nmol/min in healthy young men without significantly altering blood 5-HT concentrations (788–813 nmol/L), indicating that exogenous 5-HTP-derived 5-HT is primarily eliminated via urine. This suggests plasma 5-HT concentrations may not correlate with melatonin changes. The current study' s daytime 5-HT concentrations (320–285 ng/mL) support this conclusion, though urinary 5-HT excretion was not measured and warrants future investigation.

3.4 Effects on Plasma Melatonin Concentrations

In mammals, melatonin is synthesized primarily in the pineal gland and intestine, with other tissues producing minor amounts. Only the pineal gland and retina exhibit rhythmic secretion patterns [21-22]. Circadian melatonin rhythms feature low daytime and high nighttime concentrations [23]. In Jining Grey goats, plasma melatonin concentrations were (11.44±1.77) pg/mL and (17.61±2.53) pg/mL at 12:00 and 18:00 in spring, respectively, increasing to (72.25±5.08) pg/mL and (66.20±4.49) pg/mL at 00:00 and 03:00 [24]. Small-tailed Han, Tong, and Tan sheep exhibit seasonal variations, with lower concentrations during spring and autumn equinoxes and elevated levels during summer solstice, when secretion begins increasing at sunset [25]. In the current study, control sheep showed daytime melatonin concentrations of 38.80–59.15 pg/mL and increasing trends during 23:00–05:00 (33.67–55.18 pg/mL), but lacked clear circadian rhythms, possibly due to nighttime lighting [26] or the use of Dorset ewes with weak seasonal breeding patterns during summer months.

Exogenous melatonin precursors can elevate ovine plasma melatonin. Namboodiri et al. [8] reported that intraperitoneal 5-HTP injection (20 and 200 mg/kg BW) at 07:00 increased plasma melatonin to 64.32 and 349.89 pg/mL by 12:00, compared to 23.00 pg/mL in controls. Sugden et al. [27] demonstrated increased pineal arylalkylamine N-acetyltransferase (AA-NAT) and hydroxyindole O-methyltransferase (HIOMT) activities 5 hours after 5-HTP injection. The current trial' s peak melatonin concentrations of (82.95±16.20) pg/mL (Trial Group I) and (92.72±7.46) pg/mL (Trial Group II) after afternoon feeding exceeded results from 20 mg/kg BW 5-HTP but were lower than 200 mg/kg BW doses [8], suggesting dose-dependent effects potentially mediated by conversion enzymes [27-28].

Shindo et al. [11] demonstrated rapid, extensive tissue uptake of 5-HTP using [14C] labeling. The significantly lower melatonin concentrations in trial groups at 1.5 hours after morning feeding (09:00) may reflect rapid tissue sequestration of 5-HTP, reducing plasma precursor availability and temporarily suppressing melatonin synthesis. However, from 3.0 to 6.0 hours after morning feeding (11:00–17:00), trial groups exhibited melatonin concentrations approaching nighttime levels, demonstrating that oral 5-HTP or rumen-protected 5-HTP effectively modulates daytime secretion patterns. This aligns with Namboodiri et al. [8], who reported significant plasma melatonin increases 2–5 hours af-

ter intraperitoneal 5-HTP injection during daytime. During nighttime (19:30–05:00), trial groups maintained significantly higher melatonin concentrations than controls, contrasting with Namboodiri et al. [8] who observed no significant increases after nighttime 5-HTP injection, possibly due to administration route differences.

The dynamic relationship between plasma 5-HTP and melatonin concentrations showed good consistency during daytime, with inverse "growth-decline" patterns, but poorer alignment during nighttime. Dietary 5-HTP or rumen-protected 5-HTP effectively regulates daytime melatonin secretion patterns and concentrations. Since this study only examined summer effects, seasonal comparisons warrant further investigation.

Regarding efficacy, rumen-protected 5-HTP produced higher plasma melatonin concentrations than unprotected 5-HTP at 3.0 and 6.0 hours after morning feeding (11:00, 14:00) and 1.5, 6.0, and 9.0 hours after afternoon feeding (21:00, 02:00, 05:00), demonstrating superior effectiveness.

Conclusion

Dietary supplementation with 5-HTP (50 mg/kg BW) or rumen-protected 5-HTP (111 mg/kg BW) significantly increases plasma 5-HTP concentrations and modulates daytime melatonin secretion patterns and concentrations in sheep, with rumen-protected 5-HTP demonstrating greater efficacy, while neither treatment significantly affects plasma tryptophan or 5-HT concentrations.

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Note: Figure translations are in progress. See original paper for figures.