Effects of Supplemental Tryptophan and Rumen-Protected Tryptophan on Lactation Performance, Plasma Parameters, and Milk Melatonin Content in Dairy Cows (Postprint)

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Abstract

This study aimed to investigate the effects of dietary supplementation with tryptophan (Trp) and rumen-protected tryptophan (RPTrp) on lactation performance, plasma indices, and milk melatonin (MLT) content in dairy cows. Thirty healthy Holstein dairy cows aged 3–5 years, with 2–3 parities and at (180$±$46) days in milk, were randomly divided into three groups: a control group, experimental group I, and experimental group II, with 10 cows per group. The control group was fed a total mixed ration, while experimental groups I and II were supplemented with 100 g/(d · head) of tryptophan and 220 g/(d · head) of rumen-protected tryptophan (L-tryptophan content $ $45%), respectively, based on the control diet. The preliminary period was 7 days, and the formal experimental period was 60 days. The results showed: 1) Compared with the control group, milk yield in experimental groups I and II increased by 11.78% (P<0.05) and 17.19% (P<0.01), respectively; lactose yield in experimental groups I and II was highly significantly higher than that in the control group (P<0.01). 2) Plasma growth hormone (GH) content in experimental groups I and II was highly significantly higher than that in the control group at 14:00 and 22:00 (P<0.01); at 14:00, plasma tryptophan content in experimental group II was highly significantly higher than that in the control group (P<0.01), with no significant difference in plasma melatonin content among groups (P>0.05); at 22:00, plasma tryptophan content in experimental group II was highly significantly higher than that in the control group (P<0.01), and plasma melatonin content in experimental group II was significantly higher than that in the control group (P<0.05); there were no significant differences in plasma tryptophan, 5-hydroxytryptamine (5-HT), and melatonin contents between experimental group I and the control group at 14:00 and 22:00 (P>0.05). 3) At 05:00, milk melatonin content in experimental group II was highly significantly higher than that in the control group (P<0.01); at 18:00, there was no significant difference in milk melatonin content among groups (P>0.05). 4) Plasma total antioxidant capacity (T-AOC) in experimental group I was highly significantly higher than that in the control group (P<0.01); plasma glutathione peroxidase (GSH-Px) activity in experimental group II was highly significantly higher than that in the control group (P<0.01); plasma superoxide dismutase (SOD) activity in experimental groups I and II was highly significantly higher than that in the control group (P<0.01); plasma malondialdehyde (MDA) content in experimental group II was highly significantly lower than that in the control group (P<0.01). The results suggest that supplementation with tryptophan [100 g/(d · head)] and rumen-protected tryptophan [220 g/(d · head)] can improve milk yield, plasma GH content, and antioxidant capacity in lactating dairy cows, with rumen-protected tryptophan showing better effects; supplementation with rumen-protected tryptophan [220 g/(d · head)] can increase plasma tryptophan content and nocturnal plasma melatonin content, thereby increasing milk melatonin content in dairy cows at night.

Full Text

Effects of Tryptophan and Rumen-protected Tryptophan Supplementations on Lactation Performance, Plasma Indexes and Milk Melatonin Content of Dairy Cows

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Abstract

This study investigated the effects of dietary supplementation with tryptophan (Trp) and rumen-protected tryptophan (RPTrp) on lactation performance, plasma indexes, and milk melatonin (MLT) content in dairy cows. Thirty healthy Holstein dairy cows aged 3–5 years, with 2–3 parities and at (180$±$46) days in milk were randomly assigned to three groups (n=10 per group): control, trial I, and trial II. The control group received a total mixed ration (TMR), while trial groups I and II were supplemented with 100 g/(d · head) Trp and 220 g/(d · head) RPTrp (L-tryptophan content $ $45%), respectively, on top of the TMR. The experiment consisted of a 7-day pre-trial period followed by a 60-day formal trial period. The results showed that: (1) Compared with the control group, milk yield increased by 11.78% (P<0.05) and 17.19% (P<0.01) in trial groups I and II, respectively; lactose yield was significantly higher in both trial groups (P<0.01). (2) Plasma growth hormone (GH) content at 14:00 and 22:00 was significantly elevated in both trial groups (P<0.01) compared with the control. At 14:00, plasma tryptophan content in trial group II was significantly higher than in the control (P<0.01), while plasma melatonin content did not differ among groups (P>0.05). At 22:00, plasma tryptophan content in trial group II was significantly higher (P<0.01), and plasma melatonin content was significantly elevated (P<0.05) compared with the control. In trial group I, plasma tryptophan, 5-hydroxytryptamine (5-HT), and melatonin contents did not differ from the control at either time point (P>0.05). (3) At 05:00, milk melatonin content in trial group II was significantly higher than in the control (P<0.01); at 18:00, no significant differences were observed among groups (P>0.05). (4) Plasma total antioxidant capacity (T-AOC) was significantly higher in trial group I (P<0.01), while plasma glutathione peroxidase (GSH-Px) activity was significantly elevated in trial group II (P<0.01). Plasma superoxide dismutase (SOD) activity was significantly increased in both trial groups (P<0.01), and plasma malondialdehyde (MDA) content was significantly reduced in trial group II (P<0.01). These results indicate that supplementation with tryptophan [100 g/(d · head)] or RPTrp [220 g/(d · head)] can improve milk yield, plasma GH content, and antioxidant capacity in lactating dairy cows, with RPTrp showing superior effects. Furthermore, RPTrp supplementation [220 g/(d · head)] increased plasma tryptophan content and nighttime plasma melatonin levels, thereby elevating nighttime milk melatonin content.

Keywords: tryptophan; rumen-protected tryptophan; dairy cow; lactation performance; melatonin

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Melatonin (MLT) is an indole neuroendocrine hormone secreted by the pineal gland in mammals [1]. Research has demonstrated that melatonin exhibits various biological functions, including treating insomnia, stabilizing biological rhythms, enhancing immunity, and exerting antioxidant, anti-aging, and anticancer effects [2-3]. However, synthetic melatonin may cause endocrine disorders and other side effects, and its safety remains to be verified; consequently, most countries and regions classify synthetic melatonin as a drug [4]. Therefore, producing milk naturally rich in melatonin holds both biological significance and economic value. Tryptophan (Trp), an essential functional amino acid in animals and a precursor of melatonin, is converted to melatonin in vivo through hydroxylation, decarboxylation, N-acetylation, and methylation [5]. Esteban et al. [6] found that oral administration of tryptophan to rats significantly increased brain 5-hydroxytryptophan, 5-hydroxytryptamine (5-HT), and plasma melatonin content. Eriksson et al. [7] reported a positive correlation between serum and milk melatonin content in dairy cows, with nighttime milk melatonin content increasing as plasma melatonin levels rose. These findings suggest that dietary tryptophan supplementation in dairy cows can elevate plasma tryptophan content and promote melatonin synthesis. However, tryptophan is extensively degraded in the rumen of ruminants, making direct supplementation ineffective [8]. Supplementing with rumen-protected tryptophan (RPTrp) can effectively prevent ruminal degradation [9], increase blood tryptophan availability, and enhance melatonin synthesis, thereby raising milk melatonin content. This study investigated the effects of tryptophan and RPTrp supplementation on plasma and milk melatonin content in lactating Holstein dairy cows, and explored their impacts on lactation performance, plasma hormone levels, and antioxidant indices.

1.1 Experimental Time and Location

The experiment was conducted from October to December 2016 at the Breeding Farm of Xinjiang Tianshan Animal Husbandry Bio-Engineering Co., Ltd., located in Ashili Township, Changji City, Xinjiang Uygur Autonomous Region.

1.2 Experimental Animals and Design

Thirty healthy Holstein dairy cows aged 3–5 years, with 2–3 parities and at (180$±$46) days in milk were randomly divided into three groups: control, trial I, and trial II (n=10 per group). The control group received a total mixed ration (TMR). Trial groups I and II were supplemented with 100 g/(d · head) tryptophan (purchased from CJ Indonesia, purity $ $98%) and 220 g/(d · head) RPTrp (purchased from Beijing Yahe Nutrition High-tech Co., Ltd., tryptophan content $ $45%, rumen bypass rate $ $85%), respectively, in addition to the TMR. Both supplements were divided into two equal portions and fed at 11:00 and 19:00 daily. The pre-trial period lasted 7 days, followed by a 60-day formal trial period. During the pre-trial, cow health status, mastitis incidence, and supplement acceptance were monitored. During the formal trial, tryptophan and RPTrp were mixed with a small amount of TMR and fed individually. Daily milk yield was recorded, and milk and plasma samples were collected on days 1, 30, and 60 of the trial.

1.3 Feeding Management

All experimental animals from the control and trial groups were housed in the same pen during the trial period. TMR was fed at 11:00 and 19:00 daily. Cows had free access to feed and water, and were allowed to move freely within the pen. Manure was removed regularly to maintain pen cleanliness. The composition and nutrient levels of the TMR are presented in Table 1 .

Table 1 Composition and nutrient levels of the TMR (air-dry basis), %

Ingredients	Content	Nutrient levels[2]
Corn silage	62.11	NEL/(MJ/kg)
Alfalfa hay	16.84	EE
Soybean meal	12.36	CP
Cottonseed meal	-	ADF

Ingredients	Content	Nutrient levels[2]
Limestone	-	NDF
Premix[1]	-	Ca
NaCl	-	-
$NaHCO_3$	-	-
MgO	-	-
Total	-	-

[1]Each kilogram of premix contained: Cu 3,230 mg, Zn 5,950 mg, Mn 4,850 mg, I 120 mg, Se 150 mg, Co 90 mg, VA 804,800 IU, VD_3 188,800 IU, VE 4,600 IU, nicotinic acid 800 mg.
[2]Net energy for lactation (NEL) was a calculated value (NEL = 0.0551 × DE - 0.0946), while other nutrient levels were measured values.

1.4 Sample Collection

Plasma samples were collected at 14:00 and 22:00 on days 1, 30, and 60 of the formal trial period. During feeding, cows were restrained and blood samples (15 mL per cow) were collected via the tail vein into 5 mL heparin sodium anticoagulant tubes. Samples were centrifuged at 3,500 r/min for 15 minutes. Plasma was aspirated with a pipette, aliquoted into 2 mL Eppendorf tubes (1.5 mL per tube), labeled, and stored at -20°C until analysis.

1.5 Sample Analysis Methods

Dry matter (DM) content of TMR was determined using conventional feed analysis methods [10]. Calcium content was measured by the o-cresolphthalein complexone colorimetric method [11]. Phosphorus content was determined by the phosphorus fixation method. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were analyzed using a fiber analyzer (ANKO, USA). Crude protein (CP) content was determined using a rapid nitrogen analyzer (Elementar, Germany) according to the manufacturer' s protocol.

Milk fat, protein, and lactose were analyzed using a milk composition analyzer (MilkoScan FT+, FOSS, Denmark). Milk melatonin and plasma 5-HT, melatonin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH), and prolactin (PRL) contents were determined by enzyme-linked immunosorbent assay. Plasma malondialdehyde (MDA) content and catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities, and total antioxidant capacity (T-AOC) were measured by colorimetric methods. All these analyses were performed at Beijing Huaying Biotechnology Research Institute. Plasma tryptophan content was determined using the colorimetric method described by Xie et al. [12].

1.6 Statistical Analysis

Experimental data were analyzed using the Mixed procedure in SAS 8.0. Fixed effects included treatment, time, and their interaction. The variance structure used compound symmetry covariance (CS). Data were expressed as least squares means. Differences were considered significant at P<0.05 and extremely significant at P<0.01. Multiple comparisons were performed using lsmeans.

2.1 Effects of Tryptophan and Rumen-protected Tryptophan on Milk Yield and Composition

As shown in Table 2 , milk yield in trial groups I and II increased by 11.78% (P<0.05) and 17.19% (P<0.01) compared with the control group, respectively, with no significant difference between trial groups I and II (P>0.05). Time had no significant effect on milk yield (P>0.05), and no significant interaction between group and time was observed (P>0.05). Milk fat percentage, fat yield, protein percentage, protein yield, and lactose percentage did not differ significantly among groups (P>0.05). However, lactose yield in trial groups I and II increased by 14.66% (P<0.01) and 17.96% (P<0.01), respectively, compared with the control. Milk urea nitrogen (MUN) content did not differ among groups (P>0.05). Time had an extremely significant effect on milk protein percentage and protein yield (P<0.01) but not on other milk components (P>0.05). No significant group × time interactions were observed for milk composition parameters (P>0.05).

Table 2 Effects of tryptophan and rumen-protected tryptophan supplementations on milk yield and milk composition of dairy cows (n=10)

Items	Groups	Time/d	P-value	Groups	Group×time
Milk yield/(kg/d)	20.71Aa	23.15ABb	24.27Bb	-	-
Milk fat rate/%	3.51Bb	3.48Bb	3.83Aa	-	-
Milk fat yield/(g/d)	786.28Bb	908.03Aa	758.69Bb	-	-
Milk protein rate/%	-	-	-	-	-
Milk protein yield/(g/d)	994.14Bb	-	-	-	-
Lactose rate/%	139.87Aa	172.66Aa	-	-	-
Lactose yield/(g/d)	-	-	-	-	-
MUN content/(mg/dL)	-	-	-	-	-

Values in the same row with different small letter superscripts indicate significant difference (P<0.05), and different capital letter superscripts indicate extremely significant difference (P<0.01), while the same or no letter superscripts indicate no significant difference (P>0.05). The same applies below.

2.2 Effects of Tryptophan and Rumen-protected Tryptophan on Plasma Hormone Content

As shown in Table 3 , plasma PRL content was higher in trial groups I and II than in the control, but the differences were not significant (P>0.05). Plasma GH content in trial groups I and II increased by 11.05% (P<0.01) and 14.15% (P<0.01), respectively, compared with the control, with no significant difference between the two trial groups (P>0.05). Plasma FSH and LH contents did not differ significantly among groups (P>0.05). Time had an extremely significant effect on plasma GH and FSH contents (P<0.01) but not on PRL and LH contents (P>0.05). No significant group × time interactions were observed for plasma PRL, GH, FSH, or LH contents (P>0.05).

Table 3 Effects of tryptophan and rumen-protected tryptophan supplementations on plasma hormone contents of dairy cows (n=10)

Items	Groups	Time/d	P-value	Groups	Group×time
PRL/(IU/mL)	5.89Aa	5.67B	3.81Aa	0.0003	<0.0001
GH/(ng/mL)	4.72C	4.11ABa	6.39A	-	-
FSH/(mIU/mL)	4.84Bb	-	-	-	-
LH/(mIU/mL)	5.16Bb	5.73Aa	-	-	-

Blood samples were collected at 22:00.

2.3 Effects of Tryptophan and Rumen-protected Tryptophan on Plasma Tryptophan, 5-HT, and Melatonin Content

As shown in Table 4 , at 14:00, plasma tryptophan content in trial group I did not differ from the control (P>0.05), while trial group II showed increases of 29.58% (P<0.01) and 31.59% (P<0.01) compared with the control and trial group I, respectively. Time had an extremely significant effect on plasma tryptophan content (P<0.01), and a significant group × time interaction was observed (P<0.01). Plasma 5-HT content did not differ among groups (P>0.05), and time had no significant effect (P>0.05) nor did group × time interaction (P>0.05). Plasma melatonin content did not differ among groups (P>0.05), though time had an extremely significant effect (P<0.01) without significant group × time interaction (P>0.05).

At 22:00, plasma tryptophan content in trial group I did not differ from the control (P>0.05), while trial group II showed increases of 29.63% (P<0.01) and 32.06% (P<0.01) compared with the control and trial group I, respectively. Time had an extremely significant effect on plasma tryptophan content (P<0.01), with a significant group × time interaction (P<0.01). Plasma 5-HT content did not differ among groups (P>0.05). Plasma melatonin content in trial group I did not differ from the control (P>0.05), while trial group II showed increases of 7.87% (P<0.05) and 6.51% (P<0.05) compared with the control and trial group I, respectively. Time had an extremely significant effect on both 5-HT and melatonin contents (P<0.01), but no significant group × time interactions were observed (P>0.05).

Table 4 Effects of tryptophan and rumen-protected tryptophan supplementations on plasma Trp, 5-HT, and MLT contents of dairy cows (n=10)

Items	Groups	Time/d	P-value	Groups	Group×time
14:00					
Tryptophan/(μmol/L)	33.50Bb	32.99Bb	43.41Aa	0.83	<0.0001
5-HT/(ng/mL)	33.79Bb	38.24Aa	37.87Aa	0.0003	<0.0001
Melatonin/(pg/mL)	60.37B	53.94C	65.04A	0.1045	<0.0001
22:00					
Tryptophan/(μmol/L)	33.75Bb	33.13Bb	43.75Aa	-	-
5-HT/(ng/mL)	125.69b	127.29b	135.58a	0.77	<0.0001
Melatonin/(pg/mL)	33.77Bb	152.07Bb	120.54Bb	-	-
	38.76Aa	144.19Bc	123.90Bb	-	-
	38.09Aa	167.50Aa	144.13Aa	0.2039	<0.0001

2.4 Effects of Tryptophan and Rumen-protected Tryptophan on Milk Melatonin Content

As shown in Table 5 , at 05:00, milk melatonin content in trial group I did not differ from the control (P>0.05), while trial group II showed increases of 6.69% (P<0.01) and 17.06% (P<0.01) compared with the control and trial group I, respectively. Time had an extremely significant effect on milk melatonin content (P<0.01), with a significant group × time interaction (P<0.01).

At 18:00, milk melatonin content did not differ among groups (P>0.05). Time had an extremely significant effect on milk melatonin content (P<0.01), with a significant group × time interaction (P<0.01).

Table 5 Effects of tryptophan and rumen-protected tryptophan supplementations on milk MLT content of dairy cows (n=10)

Groups	Time/d	P-value	Groups	Group×time
18.84Bb	17.17Bb	20.10Aa	-	-
18.19Bb	17.56Bb	20.37Aa	-	-
15.78ABab	15.20Bb	16.40Aa	0.27	<0.0001

2.5 Effects of Tryptophan and Rumen-protected Tryptophan on Plasma Antioxidant Indexes

As shown in Table 6 , plasma T-AOC in trial group I was significantly higher than in the control and trial group II (P<0.01), while trial group II did not differ from the control (P>0.05). Time had an extremely significant effect on T-AOC (P<0.01), with a significant group × time interaction (P<0.01). Plasma GSH-Px activity in trial group I did not differ from the control (P>0.05), while trial group II showed increases of 5.43% (P<0.01) and 6.14% (P<0.01) compared with the control and trial group I, respectively. Time had an extremely significant effect on plasma GSH-Px activity (P<0.01), but no significant group × time interaction was observed (P>0.05). Plasma SOD activity in both trial groups I and II was significantly higher than in the control (P<0.01). A significant group × time interaction was observed for plasma SOD activity (P<0.05), though time alone had no significant effect (P>0.05). Plasma CAT activity did not differ among groups (P>0.05). Time had an extremely significant effect on CAT activity (P<0.01), but no significant group × time interaction was observed (P>0.05). Plasma MDA content in trial group I did not differ from the control (P>0.05), while trial group II showed reductions of 16.05% (P<0.01) and 8.11% (P<0.01) compared with the control and trial group I, respectively. Time had no significant effect on plasma MDA content (P>0.05), and no significant group × time interaction was observed (P>0.05).

Table 6 Effects of tryptophan and rumen-protected tryptophan supplementations on plasma antioxidant indexes of dairy cows (n=10)

Items	Groups	Time/d	P-value	Groups	Group×time
T-AOC/(U/mL)	12.00Bb	13.55Aa	11.98Bb	0.0010	<0.0001
GSH-Px/(U/mL)	909.38Bb	903.23Bb	958.72Aa	-	-
SOD/(U/mL)	888.30Bb	922.17ABb	960.87Aa	-	-
CAT/(U/mL)	63.38Bb	67.47Aa	69.38Aa	-	-
MDA/(nmol/mL)	4.86Aa	4.44Aa	4.08Bb	-	-

Samples were collected at 22:00.

3.1 Effects of Tryptophan and Rumen-protected Tryptophan on Lactation Performance in Dairy Cows

Tryptophan, as a functional essential amino acid in animals, not only participates in protein synthesis but also exhibits metabolic activity. Its metabolites —including 5-HT, melatonin, nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, and niacin—regulate various physiological processes such as feeding, growth, reproduction, lactation, and antioxidant capacity [13]. Liu et al. [14] fed 20 three-month-old weaned crossbred lambs with 0, 1, 2, and 4 g/(d • head) of RPTrp (33.3% L-tryptophan content) and found that 4 g/(d • head) RPTrp significantly improved nitrogen retention and daily weight gain while reducing urinary nitrogen excretion. Hui et al. [15] reported that supplementing Inner Mongolian cashmere goats with 0, 2, 4, and 6 g/(d • head) RPTrp improved nitrogen retention and daily weight gain, and significantly increased cashmere growth rate. Kollmann et al. [16] supplemented 12 lactating

Brown Swiss cows with 500 g/(d • head) RPTrp (25% L-tryptophan content) and found that an effective tryptophan supplementation level of 125 g/(d • head) significantly increased plasma tryptophan content and milk yield. Based on these findings, the present study set the RPTrp supplementation level at 220 g/(d • head) (calculated as 45% tryptophan content in the RPTrp product), providing an effective L-tryptophan level of 100 g/(d • head). Therefore, the tryptophan group was also set at 100 g/(d • head).

In this study, milk yield increased by 14.77% and 17.19% after tryptophan and RPTrp supplementation, respectively, consistent with previous research. This may be attributed to the fact that the experimental cows were in late gestation, a period of rapid fetal growth requiring substantial nutrients. This creates a potential tryptophan deficiency, which was alleviated by RPTrp supplementation, thereby increasing intestinal tryptophan supply and improving milk yield. Additionally, RPTrp supplementation can elevate blood free tryptophan levels, increasing tryptophan transport across the blood-brain barrier and raising brain 5-HT content. As an important central neurotransmitter, 5-HT acts on the hypothalamus and pituitary to promote PRL and GH secretion, which stimulates mammary tissue and enhances milk secretion [17]. Numerous studies have shown that tryptophan metabolite niacin can increase nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate levels, enhance rumen microbial protein production, and promote nutrient utilization, thereby increasing milk yield [18]. The increased milk yield observed with tryptophan supplementation may be due to increased free tryptophan in the rumen, which enhances microbial protein synthesis and increases intestinal microbial protein supply to the animal.

The results showed that tryptophan and RPTrp supplementation did not significantly affect milk fat percentage, protein percentage, or lactose percentage, although fat, protein, and lactose yields increased. This may be because animals mobilize body reserves to maintain homeostasis, so milk component percentages may not be affected by tryptophan supplementation. The increased component yields likely resulted from higher milk production rather than direct effects of tryptophan.

Milk urea nitrogen is an important indicator for evaluating protein utilization efficiency and the balance between protein and energy intake in dairy cows. Leonardi et al. [19] added 0.07% methionine to diets with two different protein levels (16% and 18%) and found no significant effect on MUN at the same protein level. Bach et al. [20] also reported that feeding dairy cows diets with the same protein level but different amino acid compositions did not significantly affect MUN content. In this study, mean MUN content was 18.21 mg/dL with no significant differences among groups, consistent with previous findings and indicating that tryptophan or RPTrp supplementation at the same dietary protein level does not significantly affect MUN content.

3.2 Effects of Tryptophan and Rumen-protected Tryptophan on Plasma Hormone Content in Dairy Cows

PRL and glucocorticoids primarily initiate lactation, while insulin and GH maintain lactation through nutritional regulation [21-22]. Numerous studies have shown that tryptophan can increase plasma PRL and GH levels in animals. Charney et al. [23] found that intravenous tryptophan injection in healthy humans significantly increased blood PRL and GH levels. Kasuya et al. [17] reported that intravenous tryptophan administration in cattle significantly increased cerebrospinal fluid 5-HT and plasma GH levels. The present results showing increased plasma PRL and GH levels after tryptophan and RPTrp supplementation are consistent with these findings. This is primarily because tryptophan, as a 5-HT precursor, increases brain 5-HT content, which acts on the hypothalamus and pituitary to promote PRL and GH release. Additionally, Kasuya et al. [24] demonstrated that melatonin can stimulate GH secretion by acting on the hypothalamus. As a melatonin precursor, tryptophan is converted to melatonin through hydroxylation, decarboxylation, acetylation, and methylation, thereby promoting GH secretion.

Under normal physiological conditions, FSH and LH work synergistically to stimulate follicular development, maturation, and ovulation. In this study, tryptophan and RPTrp supplementation did not significantly affect plasma FSH and LH levels, possibly because the experimental cows were pregnant, maintaining stable FSH and LH levels. The effects of tryptophan on animal reproductive performance warrant further investigation.

3.3 Effects of Tryptophan and Rumen-protected Tryptophan on Plasma Tryptophan, 5-HT, and Melatonin Content in Dairy Cows

Plasma free amino acid levels reflect amino acid metabolism in animals. After dietary amino acids reach the small intestine and are absorbed, plasma free amino acid levels rise, correlating positively with the amount of amino acids reaching the small intestine. Thus, plasma free amino acid content directly reflects amino acid absorption [25]. Ma et al. [26] reported that supplementing Liaoning cashmere goats with 4 g/(d · head) RPTrp (33% L-tryptophan content) significantly increased plasma tryptophan content. Kollmann et al. [16] found that supplementing dairy cows with 500 g/(d · head) RPTrp (25% L-tryptophan content) significantly increased plasma tryptophan content during both day and night, with a more pronounced increase at night, showing a clear diurnal rhythm. In this study, RPTrp supplementation increased plasma tryptophan content by 29.58% at 14:00 and 29.63% at 22:00 compared with the control, consistent with previous results.

5-HT is a central nervous system neurotransmitter synthesized from tryptophan by tryptophan hydroxylase and decarboxylase in 5-HT neurons [27]. This study showed that tryptophan and RPTrp supplementation did not significantly affect plasma 5-HT content, and no clear diurnal rhythm was observed. Kollmann et al. [16] also reported that RPTrp supplementation did not significantly affect plasma 5-HT content in dairy cows, consistent with our results. This may be because 5-HT cannot cross the blood-brain barrier, making peripheral and brain 5-HT levels relatively independent. Numerous studies have shown that increasing plasma tryptophan content can enhance brain 5-HT synthesis, which inhibits brain activity, reduces stress, promotes sleep, and lowers maintenance requirements, thereby improving feed conversion efficiency [28-29]. However, whether plasma tryptophan content affects plasma 5-HT levels requires further investigation.

Tryptophan, as a melatonin precursor, is converted to melatonin through hydroxylation, decarboxylation, acetylation, and methylation. Esteban et al. [6] found that oral tryptophan administration to rats significantly increased brain 5-hydroxytryptophan, 5-HT, and plasma melatonin levels. Kollmann et al. [16] reported that supplementing Brown Swiss cows with 500 g/(d · head) RPTrp (25% L-tryptophan content) significantly increased nighttime plasma melatonin content without affecting daytime levels. In this study, plasma melatonin content showed minimal variation at 14:00, consistent with the lack of significant change in plasma tryptophan content, indicating that tryptophan is extensively degraded in the rumen with limited absorption in the small intestine. RPTrp supplementation did not affect plasma melatonin content at 14:00 but significantly increased it at 22:00, showing a clear diurnal rhythm with low daytime and high nighttime levels, consistent with previous research. Since tryptophan competes with large neutral amino acids (LNAA) such as tyrosine, valine, leucine, and phenylalanine for transport across the blood-brain barrier, RPTrp supplementation can increase plasma free tryptophan levels, raise the tryptophan:LNAA ratio, and increase brain tryptophan uptake, thereby promoting 5-HT and melatonin synthesis [30].

3.4 Effects of Tryptophan and Rumen-protected Tryptophan on Milk Melatonin Content in Dairy Cows

In this study, milk melatonin content ranged from 15.70 to 20.10 pg/mL, with higher levels at 05:00 than at 18:00 in all groups, mirroring plasma melatonin patterns. Ren et al. [31] reported milk melatonin content of 7-12 pg/mL during the day and 15-26 pg/mL at night, similar to our findings. Castro et al. [32] found that nighttime milk melatonin content increased with plasma melatonin levels but with a time delay. Kollmann et al. [16] showed that RPTrp supplementation increased nighttime plasma and milk melatonin content without affecting daytime levels. In this study, tryptophan supplementation did not significantly affect milk melatonin content, consistent with its lack of effect on plasma melatonin. RPTrp supplementation did not affect milk melatonin content at 18:00, consistent with the lack of change in plasma melatonin at 14:00. However, RPTrp significantly increased milk melatonin content at 05:00, consistent with the significant increase in plasma melatonin at 22:00, indicating a positive correlation between milk and plasma melatonin content. These results demonstrate that RPTrp supplementation can increase plasma melatonin content and consequently raise milk melatonin content, with milk melatonin following a similar diurnal rhythm to plasma melatonin.

3.5 Effects of Tryptophan and Rumen-protected Tryptophan on Plasma Antioxidant Indexes in Dairy Cows

This study showed that RPTrp supplementation significantly increased plasma GSH-Px and SOD activities while decreasing MDA content. Wei et al. [33] reported that increasing dietary tryptophan levels significantly increased serum SOD activity and T-AOC in Yangzhou geese. Ma et al. [34] found that dietary supplementation with 0.18% tryptophan significantly increased serum SOD activity, glutathione (GSH) content, and GSH-Px activity in yellow-feathered broilers, consistent with our results. The amino group in tryptophan's molecular structure can bind to oxidants, hindering oxidation reactions and thereby reducing serum MDA content and enhancing antioxidant capacity [35]. Additionally, tryptophan can be synthesized into melatonin. Ozturk et al. [36] showed that melatonin injection (10 mg/d) in mice for 7 days significantly increased SOD activity. Özturk et al. [37] found that melatonin could ameliorate the decline in GSH-Px activity and GSH content induced by toxic hepatitis in mice. These findings suggest that RPTrp supplementation can increase nighttime plasma melatonin content and enhance antioxidant capacity.

Conclusion

1. Supplementation with tryptophan [100 g/(d • head)] or RPTrp [220 g/(d • head)] can increase plasma GH content, milk yield, and antioxidant capacity in lactating dairy cows, with RPTrp showing superior effects.
2. Supplementation with RPTrp [220 g/(d • head)] can increase plasma tryptophan content and nighttime plasma melatonin levels, thereby elevating nighttime milk melatonin content in dairy cows.

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