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Effect of micronutrient (L-tryptophan) imprinting on growth performance, fecal consistency, and blood parameter of Hanwoo calves

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미량영양소(L-tryptophan) 대사각인이 한우 송아지의 성장특성, 분변지수 및 혈액성상에 미치는 영향

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Abstract This study investigated the effects of rumen-protected L-tryptophan supplementation (micronutrient imprinting) on the growth performance, fecal consistency, and immunity of Hanwoo calves. Ten newborn Hanwoo calves (birth weight: 29.5 ± 3.8 kg) were randomly assigned for 120 days to the following two groups: the control group fed with a basal diet (formula feed + annual ryegrass), and the treatment group fed with a basal diet + rumen-protected L-tryptophan (0.1% of formula feed). Average daily weight gain during the entire period was significantly higher in the treatment group than in the control group ($P < 0.05$). The fecal scores were significantly higher in the control group in the duration from birth to 1 month and from 2 to 3 months of age ($P < 0.05$). The plasma magnesium concentration was significantly higher in the treatment group than in the control group ($P < 0.05$), while other blood metabolites and complete blood counts were similar for both groups. The concentrations of immunoglobulin A and M showed a tendency to increase in the treatment group as compared to the control group. Thus, our results indicate that rumen-protected L-tryptophan supplementation has a positive effect on immunity and is effective in improving the fecal score and growth performance of Hanwoo calves.

요 약 본 연구는 반추위 보호 L-tryptophan의 첨가(미량영양소 대사각인)가 한우 송아지의 성장 특성, 분변 지수 및 면역력에 미치는 영향을 조사하기 위해 수행되었다. 공시동물은 한우 신생 송아지 10두(생시체중: 29.5 ± 3.8kg)를 공시하여 120일간 이용하였다. 시험구는 기초사료(배합사료 + 애뉴얼 라이그라스)를 급여하는 대조구와 기초사료 + 반추위 보호 L-tryptophan (배합사료 급여량의 0.1%)을 급여하는 처리구의 2처리로 구분하였다. 생시체중은 처리 그룹간 유사했지만, 실험 전기간 동안 일당증체량은 대조구에 비해 처리구에서 유의적으로 높았다($P < 0.05$). 분변지수는 생시-생후 1개월 및 2 – 3개월 동안 대조구에서 유의적으로 높은 분변지수(묽은 정도)를 보였다($P < 0.05$). 혈장 마그네슘 농도는 대조구에 비해 처리구에서 유의적으로 증가되었지만($P < 0.05$), 대부분의 혈중 대사물질 농도 및 혈구 성상은 처리 그룹간 유사한 수준이었다. 면역글로불린 A 및 M 농도는 대조구에 비해 처리구에서 높은 경향을 보였다. 따라서 L-tryptophan의 첨가는 한우 송아지의 면역력에 긍정적인 영향을 미치며, 성장 특성 및 분변 지수 향상에 효과적인 것으로 판단된다.

Keywords : L-tryptophan, Hanwoo Calf, Average Daily Gain, Fecal Consistency, Immunoglobulin

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1. Introduction

In a report by the Kaneene et al [1], National Animal Health Monitoring System (NAHMS) in the United States, the average calf mortality due to disease was reported to be 10.8% before weaning and 2.4% after weaning. Considering that calf diarrhea accounts for 50 – 75% of calf mortality, strengthening immunity and preventing diarrhea are essential factors for enhancing productivity. Calf diarrhea has multiple causes, including colostrum management, sanitation management, barns, herd organization, ventilation, stress, nutrition management, and environmental conditions such as exposure to one or more infectious agents. To reduce the mortality rate of newborns and raise healthy calves, it is important to minimize exposure to pathogens and at the same time enhance immunity.

Tryptophan is closely associated with the immune system of ruminants and is known to increase nutrient utilization. An aromatic amino acid, tryptophan was first isolated from the hydrolysate of casein. Several proteins contain tryptophan, but its content is low. Free tryptophan in the blood moves to the brain through the blood-brain barrier and is converted to 5-hydroxytryptamine by 5-hydroxytryptophan in the pineal gland through enzymatic action. Valente et al [2] reported that tryptophan supplementation had a significant effect on the increase in blood serotonin and kynurenine concentrations in cattle, and that blood glucose and insulin concentrations increased. Supplementation with tryptophan is known to increase protein and starch absorption in beef cattle [3], as well as serotonin, digestive hormone (cholecystokinin), melatonin, and α-amylase activity in the duodenum and pancreas, but it decreases the concentration of ghrelin [4,5].

Melatonin is a hormone secreted by the pineal gland, synthesized by sensing photoperiods such as night and day, and recognized to affect growth and reproduction. It is known that enzymes that degrade tryptophan are secreted from immune-related cells, and it has been reported that there is a deep relationship between tryptophan and the immune system. Supplementing tryptophan to newborn Holstein calves increases the number of eosinophils in their blood, and feeding serotonin promotes the mRNA expression of immune factors [6]. Yabut et al [7] reported that serotonin plays a role in increasing the initiation and potential of the immune response.

Therefore, we hypothesized that rumen-protected L-tryptophan might have an effect on the mortality of Hanwoo calves by enhancing growth performance, fecal score index, plasma metabolites, complete blood count (CBC), and immunoglobulin (IG) levels.

2. Materials and methods

2.1 Animals, treatments, and management

A total of 10 Hanwoo newborn calves (birth weight: 29.5 ± 3.8 kg) were randomly assigned to the following two groups based on the sex ratio (3 male and 2 female calves/group): control group, fed formula feed + annual ryegrass; treatment group, fed formula feed + annual ryegrass + rumen-protected L-tryptophan (0.1% of formula feed). The rumen-protected L-tryptophan used in the present study comprised 40% L-tryptophan + 60% rumen-protected fat; the fat used in coating L-tryptophan was manufactured by a mixing reaction of palm oil and calcium.

The calves were fed the experimental diets from calving to the pre-weaning age (90 days), and then for 30 days post-weaning (91-120 days of age). The calves were allocated to different groups with their dams in pens (5 × 10 m) with sawdust to a thickness of approximately 30 cm during the pre-weaning stage, and then separated from their dam post-weaning. In the

pre-weaning stages, calves freely sucked milk, and there was no access of the dam to the calf pen when feed was provided. Formula feed was provided twice daily (08:00 and 16:00), and annual ryegrass, water, and mineral blocks were freely available. Other feeding management practices were conducted in accordance with the practices of the Research Farm of Kangwon National University. The chemical compositions of the experimental diets were analyzed following the standard methods of the AOAC [8], and neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the methods described by Van Soest et al [9]. The chemical composition of the experimental diets is listed in Table 1.

Table 1. Chemical composition of the experimental diets.

Items	Formula feed	Annual ryegrass
Dry matter (%)	90.91±0.17	94.11±0.12
Crude protein (%)	18.42±0.20	4.66±0.05
Ether extract (%)	2.31±0.02	0.79±0.02
Crude ash (%)	7.01±0.11	5.16±0.04
Neutral detergent fiber (%)	33.69±0.57	72.95±0.38
Acid detergent fiber (%)	15.75±0.41	50.96±0.70

2.2 Growth performance

Body weight (BW) was measured once a month from birth to four months of age using a cattle weighbridge installed in the pen. Average daily gain (ADG) was calculated by dividing the weight gain by the number of feeding days. Dry matter intake (DMI) was calculated by measuring the quantity of residual feed per pen before morning feeding. The feed conversion ratio (FCR) was calculated using the DMI and ADG values.

2.3 Fecal consistency

Fecal consistency was evaluated every morning and scored on a scale from 1 to 4 (1 = firm; 2 = loose, pudding; 3 = very loose, no watery separation; and 4 = very watery), according to Larson et al [10].

2.4 Blood collection and analysis

Blood was collected bimonthly from birth to 4 months of age. Before feeding in the morning, blood was collected from the jugular veins of all calves using an 18-gauge needle. Blood for CBC analysis was performed using a vacuum blood vessel (Vacutainer; Becton-Dickinson, NJ) coated with EDTA-2Na and analyzed using a veterinary multi-parameter hematology analyzer (Sysmex Corp., Kobe, Japan). Blood samples for plasma metabolites and IGs were collected with a vacuum blood vessel coated with heparin and centrifuged at 1,250 × g for 20 min. After this, the supernatant was collected, and 1 mL of each sample was divided into two microtubes and stored at -70 °C until analysis. Plasma metabolites were analyzed using an automatic blood analyzer (Hitachi 7020, Hitachi Ltd., Tokyo, Japan). IG G, A, and M were analyzed using a bovine IG ELISA kit (MYBIOSCIENCE, San Diego, CA, USA).

2.5 Statistical analysis

All results of the present study were analyzed by t-tests using the least significant difference procedure of the SAS package program (release. 9.1.3 version, 2005). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1 Growth performance

The effects of rumen-protected L-tryptophan supplementation on the growth performance of Hanwoo calves are shown in Table 2. Initial BW, final BW, ADG, DMI, and FCR were similar between the treatments from 1st to 3rd period. At the 4th period, the final BW and FCR were significantly higher in the treatment group than in the control group ($p<0.05$). For the entire

period, initial BW was similar between two groups; however, the final BW and ADG of the treatment group were significantly higher than those of the control group ($p<0.05$). The DMI did not show significant differences; thus, the FCR was significantly higher in the control group than in the treatment group ($p<0.01$).

Table 2. Effects of the supplementation of rumen-protected L-tryptophan on the growth performance of Hanwoo calves

Items	CON[1]	TRT[2]	SEM[3]	Pr>F
1st period (birth − 1 months of age)				
Initial BW (kg)	28.8	30.1	1.216	0.641
Final BW (kg)	48.1	49.7	2.130	0.715
ADG (kg/d)	0.69	0.72	0.082	0.896
DMI (kg)	0.11	0.10	0.001	0.989
FCR	0.18	0.15	0.019	0.560
2nd period (1 − 2 months of age)				
Initial BW (kg)	48.1	49.7	2.130	0.715
Final BW (kg)	62.3	69.8	2.526	0.119
ADG (kg/d)	0.30	0.43	0.035	0.051
DMI (kg)	0.47	0.49	0.045	0.884
FCR	1.80	1.12	0.208	0.081
3rd period (2 − 3 months of age)				
Initial BW (kg)	62.3	69.8	2.526	0.119
Final BW (kg)	77.3	85.6	3.063	0.165
ADG (kg/d)	0.54	0.56	0.029	0.653
DMI (kg)	1.13	1.03	0.094	0.212
Formula feed	0.65	0.67	0.068	0.926
Annual ryegrass	0.38	0.46	0.032	0.892
FCR	2.12	1.89	0.123	0.357
4th period (3 − 4 months of age)				
Initial BW (kg)	77.3	85.6	3.063	0.165
Final BW (kg)	110.4[b]	125.7[a]	4.173	0.042
ADG (kg/d)	0.79	0.96	0.051	0.077
DMI (kg)	3.84	4.10	0.096	0.212
Formula feed	3.20	3.45	0.098	0.245
Annual ryegrass	0.64	0.65	0.060	0.816
FCR	5.41[a]	4.02[b]	0.382	0.045
Entire period (birth − 4 months of age)				
Initial BW (kg)	28.8	30.1	1.360	0.641
Final BW (kg)	110.4[b]	125.7[a]	4.173	0.042
ADG (kg/d)	0.54[b]	0.64[a]	0.028	0.029
DMI (kg)	1.78	1.89	0.211	0.707
Formula feed	1.25	1.32	0.182	0.734
Annual ryegrass	0.53	0.57	0.049	0.720
FCR	3.54[a]	2.78[b]	0.143	0.009

[a,b]Means followed by different letters in the same row are significantly different ($p<0.05$).
[1]CON: control group (formula feed + annual ryegrass); [2]TRT: treatment group (formula feed + annual ryegrass + 0.1% rumen-protected L-tryptophan); [3]SEM: standard error of mean.

3.2 Fecal consistency

The effects of supplementation with rumen-protected L-tryptophan on the fecal consistency of Hanwoo calves are shown in Table 3. The fecal score index was 3.01 in the control group and 2.96 in the treatment group,

respectively, and was significantly higher in the control group than in the treatment group ($p<0.05$). In contrast, at 1 − 2 months of age, the fecal score index of the control group was lower than that of the treatment group; however, the difference was not significant. At 2 − 4 months, the fecal score index of the control group was higher than that of the treatment group. In particular, the control group showed significantly higher fecal scores than that of treatment group at 2 − 3 months ($p<0.05$).

Table 3. Effects of the supplementation of rumen-protected L-tryptophan on the fecal score index1 of Hanwoo calves

Items	CON[2]	TRT[3]	SEM[4]	PR>F
Birth − 1 mo of age	3.01[a]	2.96[b]	0.062	0.046
1 − 2 mo of age	2.06	2.13	0.117	0.060
2 − 3 mo of age	2.11[a]	2.02[b]	0.104	0.010
3 − 4 mo of age	2.14	2.13	0.129	0.706

[a,b]Means followed by different letters in the same row are significantly different ($p<0.05$).
[1]Fecal score index: 1 = normal, 2 = loose, pudding, 3 = very loose, no watery separation, and 4 = very watery; [2]CON: control group (formula feed + annual ryegrass); [3]TRT: treatment group (formula feed + annual ryegrass + 0.1% rumen-protected L-tryptophan); [4]SEM: standard error of mean.

3.3 Plasma metabolites, CBC and IG

The effects of supplementation of rumen-protected L-tryptophan on the concentration of plasma metabolites in Hanwoo calves are shown in Table 4. Supplementation with rumen- protected tryptophan showed a tendency to increase glucose, blood urea nitrogen, albumin, total protein, and triglyceride, and decrease aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT); however, there was no significant difference. The plasma magnesium concentration during the

Table 4. Effect of the supplementation of rumen-protected L-tryptophan on concentrations of plasma metabolites of Hanwoo calves

Items	Initial (birth)				Final (4 months of age)			
	CON[1]	TRT[2]	SEM[3]	Pr>F	CON	TRT	SEM	Pr>F
Glucose (mg/dL)	115.00	103.00	6.334	0.349	92.50	104.75	4.580	0.167
NEFA[4] (uEq/L)	129.00	138.00	30.058	0.887	68.50	90.50	8.335	0.174
BUN[5] (mg/dL)	7.23	11.33	1.265	0.078	14.75	16.38	0.981	0.418
Albumin (g/dL)	3.20	3.20	0.049	1.000	3.38	3.45	0.047	0.437
Total protein (g/dL)	6.08	5.63	0.200	0.258	5.70	5.88	0.122	0.486
Creatinine (mg/dL)	1.08	1.08	0.034	1.000	1.25	1.23	0.049	0.809
Cholesterol (mg/dL)	122.25	106.75	10.083	0.454	181.25	151.25	13.195	0.252
Triglyceride (mg/dL)	30.25	24.00	4.264	0.476	18.50	26.00	2.701	0.148
AST (IU/L)	86.25	51.75	12.694	0.200	99.25	80.50	8.627	0.312
ALT (IU/L)	15.50	8.00	2.257	0.068	18.50	15.00	1.735	0.316
GGT (mg/dL)	43.75	34.25	9.932	0.647	16.00	13.00	1.895	0.440
IP[6] (mg/dL)	9.50	9.85	0.606	0.783	7.45	7.53	0.253	0.888
Calcium (mg/dL)	9.28	8.93	0.125	0.142	8.68	8.90	0.117	0.341
Magnesium (mg/dL)	1.88	1.83	0.045	0.595	1.88[b]	2.28[a]	0.106	0.029

[a,b]Means followed by different letters in the same row are significantly different ($p<0.05$).
[1]CON: control group (formula feed + annual ryegrass); [2]TRT: treatment group (formula feed + annual ryegrass + 0.1% rumen-protected L-tryptophan); [3]SEM: standard error of mean; [4]NEFA: non-esterified fatty acid; [5]BUN: blood urea nitrogen; [6]IP: inorganic phosphorus.

Table 5. Effect of rumen-protected L-tryptophan on changes in CBC and IG of Hanwoo calves

Items	Initial (birth)				Middle (2 months of age)				Final (4 months of age)			
	CON[1]	TRT[2]	SEM[3]	Pr>F	CON	TRT	SEM	Pr>F	CON	TRT	SEM	Pr>F
WBC[4] (10^3/uL)	9.94	10.76	1.204	0.769	9.89	9.63	0.420	0.772	9.03	9.29	0.368	0.734
RBC[5] (10^6/uL)	8.64	7.50	0.332	0.085	8.29	6.71	0.557	0.138	7.82	7.77	0.451	0.958
Hemoglobin (g/dL)	11.73	11.73	0.586	0.995	14.05	13.30	0.686	0.599	12.13	12.63	0.453	0.596
Hematocrit (%)	38.55	33.67	1.559	0.127	39.03	31.93	2.505	0.138	35.20	35.58	1.894	0.925
Segment (%)	65.00	64.33	5.073	0.955	37.28	38.55	4.654	0.896	6.03	5.28	1.232	0.772
Lymphocyte (%)	29.80	31.40	4.381	0.875	56.70	53.40	4.413	0.721	78.43	82.08	1.111	0.073
Monocyte (%)	2.40	3.20	0.753	0.644	4.18	6.18	0.766	0.179	13.43	10.85	1.423	0.373
Eosinophil (%)	1.95	0.37	0.523	0.144	0.75	0.55	0.218	0.660	1.50	1.18	0.679	0.820
Basophil (%)	0.85	0.70	0.074	0.360	1.10	1.33	0.256	0.674	0.63	0.63	0.056	1.000
IgG (mg/dL)	1.50	1.00	0.175	0.134	1.00	1.00	0.001	1.000	1.00	1.00	0.001	1.000
IgA (mg/dL)	1.00	1.00	0.001	1.000	4.25	4.50	0.281	0.670	2.25	3.00	0.281	0.168
IgM (mg/dL)	1.25	1.00	0.134	0.356	1.00	1.00	0.001	1.000	1.00	1.50	0.175	0.134

[1]CON: control group (formula feed + annual ryegrass); [2]TRT: treatment group (formula feed + annual ryegrass + 0.1% rumen-protected L-tryptophan); [3]SEM: standard error of mean; [4]WBC: white blood cell; [5]RBC: red blood cell.

initial period was similar between treatments, but at the end of the treatment period, it was significantly increased in the treatment group compared to the control group ($p<0.05$).

The effect of supplementation of rumen-protected L-tryptophan on the CBC and IG of Hanwoo calves is shown in Table 5. CBC and IG levels did not differ between the treatment groups; however, concentration of IgA were tended to increase at control group with the passage of experimental period. IgM concentration were 1.00 and 1.50 in the control and treatment group at 2 months of age, respectively and were tended to increase in the treatment group when compared with those in the control group.

4. DISCUSSION

The digestibility of starch in the small intestine of ruminants is known to be very low at 35 – 60% [11] due to the low secretion and activity of α-amylase in the small intestine. Starch is an important energy source that accounts for most of the total digestible nutrient of feed and is a major component of corn and wheat, the main ingredients of feed. In this study, ADG and FCR significantly increased for the entire period (birth – 4 months) in the treatment group (Table 2). These results were consistent with those of a previous study showing that L-tryptophan supplementation increased the ADG and FCR of growing calves [12]. These results are considered to be due to the effect of L-tryptophan, which increases nutritional degradability, especially that of starch. According to a study by [5], L-tryptophan supplementation increases the concentration of melatonin, cholecystokinin-8, and α-amylase activity in the blood, thereby increasing starch digestibility. Cholecystokinin-8, which is affected by L-tryptophan, enhances protein digestion in the duodenum by degrading chain peptides from the abomasum [13]. In this study, the concentrations of gastrointestinal hormones and digestive enzymes were not investigated; however, although the feed intake of Hanwoo calves was similar between the treatment groups, the increase of ADG and FCR in the treatment group is thought to be due to the increased secretion of gastrointestinal hormones and digestive enzymes.

In the present study, L-tryptophan supplementation improved the fecal score index of Hanwoo calves. This result is considered to be due to the immune-enhancing effects of L-tryptophan. L-tryptophan stimulates the thymic lymphatic system to induce the proliferation and differentiation of T cells and maintain immunity by inducing a large number of lymphokines [14]. In addition, L-tryptophan acts as a protective substance in the rumen by regulating rumen pH, which is one of the important causes of diarrhea in ruminants, thereby maintaining the activity of microorganisms and preventing acidosis [15]. L-tryptophan induces the proliferation and differentiation of T cells and maintains immunity through an increase in lymphokines [14]. Calf immunity and diarrhea are affected by stress and behavioral characteristics. End products of L-tryptophan, such as melatonin, serotonin, and niacin, are known to reduce stress in animals. Choi et al [16] found that supplementation with L-tryptophan minimized stress effects and reduced the incidence of diarrhea under heat stress conditions.

Plasma metabolites did not show significant results in most of the items after L-tryptophan supplementation, but plasma magnesium levels were significantly increased. Plasma magnesium levels are affected by magnesium intake [17]. In this study, the feed intake tended to increase with the supplementing of L-tryptophan; therefore, it is considered that the concentration of magnesium was also affected. According to a

study by Lee et al [12], L-tryptophan supplementation had the effect of increasing feed intake. In other studies, L-tryptophan supplementation lowered palatability, leading to decreased intake [18]. The difference between these research results is presumed to be due to the difference in palatability of the coating materials and the way that feed additives are produced, rather than any problem with L-tryptophan itself. In addition, the increase in melatonin and serotonin, which use L-tryptophan as a precursor, reduced stress in calves, and it is thought that the intake was also effective in this regard.

We observed that CBC was not affected by supplementation of rumen-protected L-tryptophan. Similarly, Lee et al [12] found no changes in WBC, LYM, GRA, RBC, HGB, HCT, mean corpuscular hemoglobin, or PLT of steers fed with 0.1% rumen-protected L-tryptophan.

In this study, IG concentration showed a tendency to increase, although the difference was not statistically significant. Tryptophan can promote the secretion of IgG by generating inflammatory cytokines and affecting the secretion of T and B cells [19]. Additionally, supplementation of L-tryptophan to cows has been shown to increase the IgG content in milk [20]. Therefore, similar to previous studies, the results of this study are thought to have little effect on the increase in IgG levels.

5. Conclusion

Supplementation with rumen-protected L-tryptophan improved the ADG and FCR of Hanwoo calves. Therefore, the present results indicate that supplementation with rumen-protected L-tryptophan has a positive effect on the growth performance of Hanwoo calves. In addition, our results suggest that rumen-protected L-tryptophan may be effective in increasing income by reducing calf diarrhea and calf mortality rates.

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