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Effect of dietary rumen-protected L-tryptophan supplementation on growth performance, blood hematological and biochemical profiles, and gene expression in Korean native steers under cold environment

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Simple Summary: In this study, the effect of dietary rumen-protected L-tryptophan (RPT) supplement on growth performance, blood hematological and biochemical profiles, and gene expression was investigated in beef steers during cold environment. We revealed that supplementation of 0.1% RPT incorporated into diet was beneficial owing to enhanced growth performance by increasing ADG and glucose level, decreasing feed conversion ratio, and maintaining homeostasis in immune responses in beef steers during cold environment.

Abstract: We assessed the growth performance, physiological traits, and gene expressions in steers fed with dietary rumen-protected L-tryptophan (RPT) under cold environment. Eight Korean native steers were assigned to two dietary groups, no RPT (Control) and RPT (0.1% RPT supplementation on a dry matter basis), 6 wks. Maximum and minimum temperatures throughout the experiment were 6.7°C and -7.0°C, respectively. Supplementation of 0.1% RPT to a total mixed ration did not increase body weight but had positive effects of elevating average daily gain (ADG) and reducing the feed conversion ratio (FCR) at day 27 and 48. Metabolic parameter showed higher glucose level (at day 27) in the 0.1% RPT group compared to the control group. Real-time PCR analysis showed no significant differences in the expression of muscle (MYF6, MyoD, and Desmin) metabolism genes between the two groups, whereas the expression of fat (PPARγ, C/EBPα, and FABP4) metabolism genes was lower in the 0.1% RPT group than in the control group. Thus, we demonstrate that long-term (6 wks) dietary supplementation of 0.1% RPT was beneficial owing to enhanced growth performance by increasing ADG and glucose level, decreasing FCR, and maintaining homeostasis in immune responses in beef steers during cold environment.

Keywords: rumen-protected L-tryptophan; growth performance; metabolites; glucose; gene expression

1. Introduction

The climate in the Korean peninsula is becoming more polarized owing to the global climate change resulting in longer colder winters and longer hotter summers [1,2]. Given this inevitable phenomenon, the decline in productivity of ruminants resulting in serious economic effects due to low temperature in winter is expected to accelerate [3,4]. Therefore, it is pivotal to establish a nutritional strategy that helps to minimize the impaired productivity of beef cattle due to temperature conditions. Cold stress causes negative effects on growth performance and immune cell population by influencing metabolic and immunological activities [5,6]. Thus, hematological profiles, if measured, could be a good indicator of how stressors affect immune function of the body [6,7]. The thermo-neutral zone for growing Korean native beef cattle is approximately 4 to 20°C and the critical temperature is approximately -10°C [1,3]. In Korea, the temperature during Nov to Feb often falls below -20°C at nights and fluctuates from approximately -5°C to -15°C during daytime. Given this, adaptation to cold environments in ruminants involves increasing thermal insulation, appetite, and basal metabolic intensity that improve cold hardiness and reduce the risks of both acute and chronic cold stress [5,6,8]. Cold stress affects beef cattle production by elevating resting heat production, energy requirement for maintenance, and appetite drive, while decreasing feed digestibility. The appetite stimulation may partially counteract the increased energy requirement, but it cannot fully alleviate the reduced efficiency of utilization of dietary energy.

L-tryptophan (TRP) is known to play a pivotal role in metabolic, physiological, and organ development and growth [9,10]. Lack of TRP may adversely affect feed intake and growth performance. However, in most ruminants, TRP is not deficient due to ruminal fermentation [9,11]. In particular, TRP in ruminants has been reported to increase the secretion and activity of pancreatic α-amylase, thereby increasing starch digestibility by promoting the secretion of the intestinal hormone cholecystokinin (CCK) [9,12,13]. TRP is also known as a constituent of niacin, a precursor of serotonin and melatonin, and to have antioxidative and stress relieving properties [9,11]. Thus, TRP is proposed as an essential amino acid (AA) that can help avert the decline in animal productivity due to low temperature environment under climate change. On the other hand, the amounts of essential AAs such as TRP supplied by microbes are sufficient only to support maintenance and normal milk and beef production and not maximal animal growth. Thus, in order to ensure the highest possible productivity in ruminants, it is essential to supply more AAs, in particular TRP, to meet the requirement.

Feeding dietary rumen-protected L-tryptophan (RPT) has been shown to not only increase growth performance of lambs [14] and wool output in sheep [15] but also improve N utilization in goats [9,16] and average daily gain (ADG) and N utilization in cashmere goats [11]. However, the effect of RPT on performance, blood hematology, and biochemistry and related gene expression in Korean native steer under cold stress conditions is yet to be investigated. Information regarding ruminal bypass AA have been limited to a few essential AAs but not RPT. Therefore, we hypothesized that dietary supplementation of RPT may enhance growth performance, alter blood parameters, and related gene expression in Korean native steers under cold environment, which was investigated in the present study.

2. Materials and Methods

2.1. Animals and feeding trial

The experimental procedure and methods were approved by the Animal Welfare and Ethics Authority of Konkuk University, Seoul, Republic of Korea (approval no: KU18178).

Eight Korean native steers [249 ± 21.6 day-old; body weight (BW) = 279 ± 16.6 kg] were selected and randomly assigned into one of two groups: total mixed ration (TMR) without RPT (control, n = 4) and TMR with 0.1% RPT (RPT, n = 4) groups. The experiment was conducted outdoors, under a shed, at the experiment farm (Chungju, Chungcheongbuk-do, Republic of Korea). All animals were protected from the rain by covering using a ceiling. The mean weights of the animals at the starting period of experiment (at day 0) were not significantly different between the groups ($p = 0.950$). After grouping by statistical analysis of individual body weights, the animals were housed in 1.0 × 6 m^2

individual pens for the duration of the experiment. TMR (Nonghyup Feed Co., Ltd., Yangju-si, Korea), which did not contain antibiotics, was fed to animals as a basal diet. The animals were fed 10 kg TMR/day/animal to meet nutrition requirements of the National Research Council [17]. The RPT group was fed TMR mixed with RPT (0.1% of TMR as-fed basis; CJ CheilJedang, Suwon, Republic of Korea) once a day at 0800 h. The rate of by-pass is above 95% (flowing corporation data). Water was available *ad libitum*. The chemical analysis (moisture, crude protein, crude fat, contents of 19 AAs, etc.) of the feed stored at -20°C was performed (Table 1). Body weight of animals was measured three times, at day 0, 27, and 48. The ADG of the animals was calculated by dividing the difference in weight, between the initial weight and the end weight, by the total number of days during the experiment. Feed intake (FI) was calculated by examining total daily feeding and the orts amount of individual animals every morning at 0700 prior to providing fresh feed.

Table 1. Chemical composition and amino acids of the basal diet used in this study.

	TMR[1]
Chemical composition, % on a dry matter basis	
Dry matter	86.82
Crude protein	15.05
Ether extract	2.49
Crude fiber	25.90
Crude ash	6.75
Acid detergent fiber	32.87
Neutral detergent fiber	52.26
Amino acids, % on a dry matter basis	
Tryptophan	0.09
Methionine	0.09
Niacin	0.00
Lysine	0.54
Aspartic acid	1.20
Threonine	0.48
Serine	0.57
Glutamic acid	2.04
Glycine	0.61
Alanine	0.79
Valine	0.67
Isoleucine	0.47
Leucine	0.97
Tyrosine	0.29
Phenylalanine	0.55
Histidine	0.29
Arginine	0.71
Cystine	0.12
Proline	0.91

[1] TMR, total mixed ration.

2.2. Environmental qualifications and measurements

Temperature (°C) and relative humidity (%) were recorded during the experiment period with a portable temperature and humidity meter (MHT-381SD, Lutron Co., LTD., Taiwan) at hourly intervals (Table 2). During days 7 to 13 and 42 to 48, minimum and maximum temperatures were -7.0°C and 6.7°C, respectively. Total average temperature during the experiment period (days 7 to 48)

was -9.5°C and was lower than the adaptation period (days 0 to 6). The average temperature during adaptation period was 1.3°C.

Table 2. Changes in the temperature and relative humidity during the experiment period.

	Temperature, °C			Relative humidity, %		
Period, days	**Min**	**Max**	**AVG[1]**	**Min**	**Max**	**AVG**
0 to 6[2]	-2.4	9.7	1.3	19.4	94.2	69.4
7 to 13	-15.7	13.4	-7.0	12.2	78.1	45.3
14 to 20	-14.0	13.5	-5.4	10.3	85.9	45.6
21 to 27	-14.6	15.8	-3.2	12.1	87.1	51.3
28 to 34	-7.3	79.8	1.9	17.9	85.3	45.9
35 to 41	-6.9	22.0	3.7	17.9	89.8	50.8
42 to 48	-5.9	23.5	6.7	15.8	89.9	61.9
7 to 48	-9.5	16.8	-0.3	15.1	87.2	52.9

[1] AVG, Average of temperature and relative humidity during day and nighttime for 7 days. [2] Days 0 to 6, adaptation period.

2.3. Blood collection and analysis

Blood samples were collected three times at day 0, 27, and 48 of the experiment period at 1400 h from jugular venipuncture into K_2 EDTA-treated vacutainers (4-mL; Becton Dickinson, Franklin Lakes, NJ, USA) for measuring hematology (white blood cells, lymphocytes, monocytes, granulocytes, red blood cells, hemoglobin, hematocrit, mean corpuscular hemoglobin, and plateletcrit) using the VetScan HM2 analyzer (Abaxis, Union City, CA, USA). Subsequently, blood in serum tubes was centrifuged at 2,500 × g for 15 min at 4°C. The serum was used for measurement of biochemistry parameters (glucose, total protein, blood urea nitrogen, albumin, and triglyceride) using the automated biochemical analyzer (Hitachi Automatic Analyzer model 7180, Hitachi, Tokyo, Japan) according to manufacturer's instructions.

2.4. Tissue collection and analysis

Longissimus dorsi muscle sample (12 to 13 ribs) for each steer was collected by biopsy at the final day of the experiment (day 48) using a spring-loaded biopsy instrument (Biotech, Nitra, Republic of Slovakia). The tissue samples were washed in Diethylpyrocarbonate (D5758, Sigma Aldrich, St. Louis, MO, USA) treated and autoclaved water, transferred into a 2-mL tube, and then incubated in a liquid nitrogen gas locker until analysis. The tissue samples (2 g) were ground into powder under freezing conditions, with liquid nitrogen, and RNA was extracted from each tissue sample as previously described [18]. The concentration of RNA was determined by spectrophotometric analysis (NanoDrip 1000, Thermo Scientific, Seoul, Republic of Korea). The RNA integrity was estimated using an RNA Nano 6000 Assay Kit for an Agilent Bioanalyzer 2100 system (Agilent Technologies, Inc., Richardson, USA). When the number of RNA integrity (RIN) was more than 6, complementary DNA (cDNA) synthesis was performed. RIN in the current study was 6.5 ± 0.27. RNA was used for real-time PCR analysis.

To synthesize cDNA in the procedure of real-time PCR analysis, 1 μg of RNA was reversely transcribed in a 100 μL reaction volume with an iScript™ cDNA synthesis kit (Bio-Rad Laboratories, Inc., CA, USA) according to manufacturer's instructions. Quantitative real-time PCR was performed on duplicate samples by using a CFX Connect™ Real-Time System (Bio-Rad Laboratories, Inc.) with IQ™ SYBR Green Supermix (Bio-Rad Laboratories, Inc.) reagents. The following PCR conditions were used: 95°C for 3 min and 40 cycles at 95°C for 10 s, 51°C to 65°C for 30 s and 72°C for 30 s. Specific primer of genes involved in muscle (MYF6, MyoD, and Desmin) and fat (PPARγ, C/EBPα, and FABP4) is shown in Table S1. Triple housekeeping genes (18S, GAPDH, and RPLP0) were used as the

internal controls. Primer sequences used for quantitative reverse transcription PCR assay are presented in Table S1.

2.5. Statistical analysis

Data sets were analyzed using the MIXED procedure of SAS (version 9.0; SAS institute Inc., Cary, NC). Whole blood for hematological analysis was collected three times (at day 0, 27, and 48). This data was used for repeated measurement analysis to investigate the interaction effect (treatment × day). Growth performance including BW, ADG, and feed conversion ratio (FCR), and mRNA expression were assessed using the Student`s t-test by using JMP 5.0 software package (SAS Institute Inc., Cary, NC, USA). The Tukey test was used to compare the differences between treatment means. The normality of the data distribution was tested prior to final comparison by SAS. Statistical differences were considered significant at $p < 0.05$.

3. Results

3.1. Growth performance during cold temperature

We investigated performance parameters of ADG, FCR, and feed intake of steers supplemented with TMR containing 0.1% RPT during cold environment. As shown in Table 3, higher ADG and lower FCR (both $p < 0.05$) in the RPT group compared with the control group were observed at day 27 of the experiment (Table 3). In addition, animals in the RPT group showed higher ($p = 0.001$) ADG and lower ($p = 0.001$) FCR compared with the control group during the final day of the experiment.

Table 3. Effect of 0.1% L-tryptophan (RPT) supplementation to total mixed ration (TMR) on growth performance in cattle during cold environment.

	Control[1]	RPT[2]	SEM[3]	*P*-value
Day 0				
Body weight, kg	278.5	279.7	7.69	0.950
Day 27				
Body weight, kg	285.1	301.5	8.30	0.360
ADG[4], kg/day	0.224	0.753	0.130	0.030
Feed conversion ratio[5]	44.4	13.3	13.25	0.035
Day 48				
Body weight, kg	290.9	315.7	4.72	0.194
ADG, kg/day	0.262	0.766	0.1	<0.001
Feed conversion ratio	13.03	4.82	1.18	<0.001

Values are expressed as means (n = 4). [1] Control, no RPT supplementation to TMR. [2] RPT, 0.1% RPT supplementation to TMR. [3]SEM, standard error of the mean. [4] ADG, body weight gain against initial body weight/experimental days. [5] Feed conversion ratio, ratio of total feed intake *versus* total weight gain.

In the observation of feed intake (FI) in steers during cold temperature, there was no difference in FI between the two groups during adaptation period (days 0 to 6); However, the FI was higher ($p = 0.038$) in the RPT group than in the control group during the final week (Table 4). Average FI in the RPT group was higher ($p = 0.005$) compared to that in the control group.

Table 4. Effect of 0.1% L-tryptophan (RPT) supplementation on feed intake in cattle during cold environment.

Period, days	Control[1]	RPT[2]	SEM[3]	*P*-value
0 to 6[4]	8.93	8.56	0.108	0.088
7 to 13	9.24	9.21	1.647	0.948
14 to 20	9.41	9.56	1.171	0.623
21 to 27	9.60	9.68	1.200	0.791
28 to 34	9.68	10.22	1.557	0.201
35 to 41	9.15	10.02	0.743	0.063
42 to 48	9.54	10.56	0.725	0.038
7 to 48	9.43	9.86	1.533	0.011

Values are expressed as means (n = 4). [1] Control, no RPT supplementation to total mixed ration. [2] RPT, 0.1% RPT supplementation to TMR. [3] SEM, standard error of the mean. [4] Days 0 to 6, adaptation period.

3.2. Physiological parameters in blood during cold temperature

Blood hematological and biochemical parameters were measured to determine the physiological conditions of steers supplemented with TMR containing 0.1% RPT during cold environment (Table 5).

Table 5. Hematological and biochemical analyses of blood in steers supplemented with TMR containing 0.1% RPT during cold environment.

	Control[1]			RPT[2]			SEM[3]	*P*-value		
Items[4]	D 0	D 27	D 48	D 0	D 27	D 48		Treatment (T)	Days (D)	T x D
Hematological parameters										
WBC	9.28	9.44	8.92	8.66	8.70	9.34	0.335	0.6815	0.9841	0.7830
LYM	6.07	6.25	5.26	5.55	6.55	5.98	0.234	0.7329	0.5086	0.3023
MON	0.07^{b}	0.08^{b}	0.56^{a}	0.06	0.07	0.12	0.043	0.0047	0.0002	0.0019
GRA	2.98	2.94	3.44	3.01	2.09	3.29	0.214	0.4665	0.3074	0.4322
RBC	10.33	9.97	9.00	10.70	10.34	10.08	0.266	0.2118	0.2050	0.6769
HGB	13.33	13.73	12.65	13.60	14.20	13.68	0.237	0.2449	0.9520	0.4339
HCT	34.57	34.12	31.66	35.00	34.64	33.88	0.584	0.3946	0.3787	0.7956
MCH	13.03	13.85	14.10	12.83	13.80	13.70	0.248	0.6806	0.2577	0.9625
PLT	398.5	455.8	367.5	378.0	476.8	379.5	15.39	0.8862	0.0360	0.8263
Biochemical parameters										
Glucose	84.0	87.0	78.5	77.0^{B}	86.3^{A}	74.3^{B}	1.32	0.0534	0.0015	0.4345
TP	6.28	6.28	6.05	6.40	6.48	6.20	0.055	0.1524	0.1444	0.9577
BUN	15.68	19.65	15.35	15.60	18.95	16.05	0.527	0.9773	0.0036	0.8052
Albumin	3.65	3.58	3.53	3.50	3.60	3.45	0.033	0.3378	0.4384	0.5805
TG	13.3	13.3	13.0	11.8	18.8	11.3	1.09	0.7354	0.3034	0.3293

Values are expressed as means (n = 4). [a,b, A,B] indicate significant differences compared to the initial period in the control and RPT groups ($p < 0.05$, Turkey test). [1] Control, no RPT TMR. [2] RPT, 0.1% RPT supplementation to TMR. [3] SEM, standard error of the mean. [4] Abbreviations: WBC, white blood cell; LYM, lymphocyte; MON, monocyte; GRA, granulocyte; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCH, mean corpuscular hemoglobin; PLT, platelet; TP, total protein; BUN, blood urea nitrogen; TG, triglyceride.

Our results showed that 0.1% RPT did not cause blood hematological changes except for monocytes (MON; $p = 0.005$). Compared to the control group during the adaptation period (day 0), a higher value of MON in the control group was observed at day 48 ($p < 0.05$). In contrast, animals in the 0.1% RPT group did not show any changes in the value of blood MON during whole experiment period. All values of MON in the control group and 0.1% RPT group were within the normal range.

All blood biochemistry parameters except glucose ($p < 0.005$) demonstrated no significant differences ($p > 0.05$) between the two groups. Lack of significant differences in serum total protein (TP), blood urea nitrogen (BUN), albumin, and triglycerides (TG) revealed that 0.1% TRP did not alter the homeostasis mechanism. Expectedly, higher ($p < 0.05$) glucose level in the RPT group, compared with the control group, was observed at day 27 of the experiment period, and the glucose level returned to the normal values at day 48 of the experiment.

3.3. Relative gene expression in fat and muscle loin tissues of steers during cold temperature

We further observed changes in gene expression related to muscle and fat metabolism in loin tissues of steers supplemented with TMR containing 0.1% RPT during cold environment (Table 6).

Table 6. Relative expression of fat (PPARγ, C/EBPα, and FABP4) and muscle (MYF6, MyoD, and Desmin) genes in loin tissue of cattle supplemented with total mixed ration (TMR) containing 0.1% L-tryptophan (RPT) during cold environment.

	Control[1]	RPT[2]	SEM[3]	*P*-value
PPARγ	1.000	0.274	0.2302	< 0.000
C/EBPα	1.000	0.293	0.2714	< 0.000
FABP4	1.000	0.233	0.2618	< 0.000
MYF6	1.000	0.834	0.1667	0.071
MyoD	1.000	0.857	0.1822	0.676
Desmin	1.000	0.871	0.1902	0.140

Values are expressed as means (n = 4). Loin tissue of each cattle was used to estimate expression of the fat (PPARγ, C/EBPα, and FABP4) and muscle (MYF6, MyoD, and Desmin) genes by real-time PCR analysis. The results were normalized using GAPDH, RPLP0, and 18S as internal controls. [1] Control, no RPT TMR. [2] RPT, 0.1% RPT supplementation to TMR. [3] SEM, standard error of the mean.

In the present study, there were no differences ($p > 0.05$) in the expression of muscle metabolism genes including MYF6, MyoD, and Desmin between the two groups. However, the expression of fat metabolism genes including PPARγ, C/EBPα, and FABP4 was higher ($p < 0.0001$) in the RPT group compare to the control group.

4. Discussion

L-tryptophan (TRP) is reported to be a limiting AA in growing lambs [11,14] and cattle [9] during the process of non-protein N utilization. Thus, supplementation of TRP in a rumen-protected form has positive effects on growth performance. L-tryptophan metabolites can affect growth, development, and health of beef cattle [9]. It is also known as a precursor of a stress relieving neurotransmitter called serotonin [9,19]. Therefore, in the present study, we can postulate that animals in the RPT group were more tolerant to cold stress situation, and thus showing higher weight gain and lower FCR.

The growth hormone-releasing hormone (GHRH) somatostatin (SS) and ghrelin are known to play pivotal roles in relation to growth hormone (GH) secretion regulation and are known to be involved in the serotonin regulation in ruminants [20,21]. Despite the limitation of this study on not measuring hormones such as serotonin levels, we observed higher FI in the RPT group compared to the control group (Table 4). Ma et al. [11] supplied two dosages of rumen-protected TRP to cashmere goats and observed an increase in the final BW and ADG in the supplemented group. Higher total FI intake could also postulate the fact that the steers in the RPT group tended to consume more feed in order to receive higher amounts of RPT, enabling them to better cope with cold stress conditions during the experiment period.

Higher amounts of RPT [over 0.5% of TMR, dry matter (DM)] have been documented to cause decreased FI due to lower palatability [8,22,23]. However, no changes in FI was observed by supplementing RPT in Korean native steers in a previous study in our laboratory (unpublished data-under revision) under normal environmental conditions. In contrast, in the present study, higher FI

in the RPT group (Table 4) implies that the amount of RPT up to 0.1% did not decrease the palatability of the feed. Higher FI in the RPT group may also postulate increased need of the animals to cope with cold stress conditions during the experiment period.

Hematological parameters are important to assess the physiological status of animals and to monitor stress and pathological changes [2,6,23]. Responses to a particular stress factor are quantitatively related to the severity and longevity of the stress [2,6,7,23]. A study on evaluation of the ruminant complete blood cell count showed that MON participate in the immune response by entering the tissues from the circulation to become macrophages, capable of phagocytosis of infectious organisms, particulates, and cell debris [24]. Their population is quite variable in cattle and is not a sensitive indicator of disease processes, such as increases that may accompany chronic inflammation, tissue necrosis, hemolysis, or a stress response [25]. Low MON numbers have been associated with endotoxemia and viremia [25]. In the present study, we did not observe any difference in blood hematological parameters in both groups except for the lower values of MON in the 0.1% RPT group (Table 5). The higher value of MON in the control group at day 48 ($p < 0.05$) was comparable to that in the control group during the adaptation period (day 0). In contrast, a constant value of MON was observed in the 0.1% RTP group at day 27 and 48 under cold environment, highlighting that animals supplemented with 0.1% RPT via TMR can modulate the maintenance of host homeostasis under stress due to cold temperature.

Little is known regarding the effects of melatonin, and TRP on cattle digestive processes. In rats, *in vivo* and *in vitro* studies [12,13] have shown that the stimulatory effects of melatonin or TRP on the exocrine pancreas activity involves cholecystokinin secretion, which is known to be a regulator of the pancreatic enzyme in cattle [26]. This effect is attributed to higher release of α-amylase, which eventually increases starch digestibility in cattle [9]. As feed intake was not affected at day 27 (Table 4) in the present study, dietary RPT supplementation likely modulated the activity of the digestive enzymes, resulting in higher glucose level in the RPT group compared to the control group at day 27. Blood glucose level is one of the most common secondary stress response parameters tested, and its increase is often associated to an elevation of blood cortisol in steers under stress conditions [27]. Similarly, a study on serum biochemical parameters of sheep under different environmental temperatures showed that higher concentration of serum glucose under cold conditions (4°C) compared with under optimal temperature (21°C) and heat stress (40°C) [28]. Indeed, it is known as a constituent of niacin, a precursor of serotonin and melatonin, and to have antioxidative and stress relieving properties [9,11]. In this study, higher blood glucose and ADG of cattle in the RPT group (Tables 3 and 5) demonstrated the effects of TRP metabolites in relieving stress in these cattle.

In a previous study in our laboratory (unpublished data, under review) we observed that relative mRNA expression levels, including MYF6, MyoG, FABP4, and LPL genes, were higher in the RPT supplemented group than in the control group of Korean native steers. While the MYF6 and MyoG are representative of muscle differentiation [29], FABP4 is documented to be involved in intracellular transport and fatty acid metabolism [30,31]. Thus, 0.1% RPT alone may not be effective to alter the expression of muscle metabolism genes including MYF6, MyoD, and Desmin. However, with respect to the fat metabolism and its related genes, 0.1% RPT could decrease the expression of PPARγ, C/EBPα, and FABP4 genes. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors of nuclear hormone receptor that reduces triglyceride level and is involved in the regulation of energy homeostasis [32]. The C/EBPα gene provides instructions for making a protein called CCAAT enhancer-binding protein alpha. This protein is a transcription factor, which means that it attaches (binds) to specific regions of DNA and helps to control the activity (expression) of certain genes. FABP4 encodes the fatty acid binding protein found in adipocytes [30]. Fatty acid binding proteins are a family of small, highly conserved, cytoplasmic proteins that bind long-chain fatty acids and other hydrophobic ligands. It is thought that FABPs roles include fatty acid uptake, transport, and metabolism. The decrease in gene expression of PPARγ, C/EBPα, and FABP4 in the present study may be attributed to muscle tissues that utilized free fatty acid for myocyte differentiation. This phenomenon could be caused by direct involvement of TRP as a blocking block or by indirect involvement of metabolic components related to TRP or both. This phenomenon

suggests that dietary supplementation of RPT enhances muscle differentiation and intracellular transportation of fatty acids by inhibiting the catabolism of fat in muscle. The intramuscular fat and intramuscular fatty acid concentration are important in meat quality improvement [33,34]. It has been stated that peroxisome proliferator-activated receptor γ (PPARγ) is the pivotally important gene in relation to lipid metabolism in muscle tissue [35]. Precedent studies reported the effects of different dietary energy levels on intramuscular deposition and fatty-acid composition in beef cattle [36,37]. Most recently, Yang et al. [38] investigated the effects of diets with different energy levels on fat deposition and the fatty acid profile of the *longissimus dorsi* muscle in yak and the role of genes involved in lipid metabolism in changing the fatty acid composition. They concluded that the high energy diets promoted the deposition and partial fatty acid content of *longissimus dorsi* muscle mainly by up-regulation of mRNA expression of ACACA, SCD, FASN, SREBP-1c, PPARγ and FABP4. However, in the present study, since the energy and amino acid are adversely related, up-regulation of mRNA expression of PPARγ, C/EBPα and FABP4 could be seen in non-supplemented RPT group (control) which is in line with the aforementioned study. Different energy or protein levels, herein supplementation of TRP, may alter intramuscular fat deposition into muscle by regulating PPARγ. PPARγ is in charge of some promotion including adipocyte proteins or enzymes such as such as fatty acid binding protein (FABP4), fatty acid synthase (FASN) and lipoprotein lipase (LPL) [39]. Since very little information is available regarding the effect of RPT on fatty acid gene expressions in *longissimus dorsi* muscle, further investigations are necessary in order to confirm these results and to bring more insights to the available knowledge.

5. Conclusions

This study indicates that long-term (6 wks) dietary supplementation of 0.1% RPT enhances growth performance in Korean native steers by modulating the immune responses and elevating glucose level under cold environment. In addition, 0.1% RPT reduced adipogenic gene expression, which may contribute to muscle tissues that utilized free fat acid for myocyte differentiation of steers during cold environment. Therefore, dietary supplementation of 0.1% RPT is beneficial in reducing the decline in productivity of beef cattle during cold stress.

Supplementary Materials: Table S1: Primer sequences used for quantitative reverse transcription PCR assay.

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Gene symbol	Gene name	Annealing temperature, °C	Primers
C/EBPα	CCAAT/enhancer-binding protein alpha	60.3	F: CCGTGGACAAGAACAGCAACGA R: GGCGGTCATTGTCACTGGTCAG
FABP4	Fatty acid binding protein 4	60.0	F: GTGTGATGCATTTGTAGGT R: CTGGTGGCAGTGACACCAT
PPARγ	Peroxisome proliferator-activated receptor gamma	61.0	F: TGGAGACCGCCCAGGTTTGC R: AGCTGGGAGGACTCGGGGTG
MyoD	Myoblast determination protein	59.6	F: AGAGTTGCTTTGCCAGAG R: CTGCCTGCCGTATAAACA
MYF6	Myogenic factor 6	60.7	F: GAAGGAGGGACAAGCATTGA R: GAGGAAATGCTGTCCACGAT
DESMIN	Desmin	61.0	F: GGACCTGCTCAATGTCAAGA R: GGAAGTTGAGGGCAGAGAAG
18S	18S ribosomal RNA	51.0	F: ACCCATTCGAACGTCTGCCCTATT R: TCCTTGGATTGTGGTAGCCGTTTCT
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	60.0	F: GGCAAGGTCATCCCTGAG R: GCAGGTCAGATCCACAACAG
RPLP0	Ribosomal protein lateral stalk subunit P0	55.0	F: CAACCCTGAAGTGCTTGACAT R: AGGCAGATGGATCAGCCA