Effects of different levels of protein supplements in the diet of early-weaned yaks on growth performance, intestinal development, and immune response to tuberculosis

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Abstract

This study was conducted to determine the effects of different levels of crude protein (CP) supplements to the diet of early-weaned yaks on their growth performance, intestinal development, and immune response. Forty 3-month-old weaned yaks were selected and assigned to four dietary groups (Control, 17, 19 and 21% CP). Dietary CP supplements had a significant effect on average daily gain (ADG), crypt depth (CD) (duodenum, jejunum and ileum), villous height (VH) (duodenum, jejunum and ileum) and CD/VH (jejunum and ileum). Average gain, CD (duodenum, jejunum and ileum) and VH (ileum) showed quadratic increases as the dietary CP increased, whereas CD/VH (jejenum and ileum) ratios showed quadratic decreases. Blood urea nitrogen (BUN), glucose (GLU), immunoglobulin G (IgG), IgM, interleukin-1 (IL-1), IL-2, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ concentrations increased significantly, whereas albumin (ALB), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) decreased significantly with dietary CP supplements. Dietary CP supplements significantly increased the concentrations of IL-6, TNF-α, IFN-γ and the nuclear factor of activated T cell transcription factor (NF-AT) for gene expression. As the dietary CP supplements increased, IL-6, IFN-γ and NF-AT gene expression showed quadratic increases. These results showed that the appropriate dietary CP supplementation improved the growth performance and intestinal development of early-weaned yaks and thus that the CP supplements were beneficial and enhanced the humoral immunity response of yaks.

Introduction

The yak (Bos gaurus) is an iconic symbol of high altitudes and of Tibet. More than 14 million domestic yaks provide the basic resources (such as meat, milk, transportation, dung for fuel and hides for tented accommodations) that are necessary for Tibetans and other nomadic pastoralists in high-altitude environments (Wiener et al., 2003). Unfortunately, bovine tuberculosis is a chronic bacterial disease of animals and humans and is a major infectious disease among cattle, other domesticated animals, and certain wildlife populations in a large number of countries (Gumi et al., 2012; Schiller et al., 2010). In Tibet, this disease remains a serious public health problem and causes significant economic losses in the production of yaks (Gao et al., 2012). Moreover, yaks must survive inadequate feeding during the long cold season (October to May), which results in malnutrition and low production (Miller, 1996), because of the herbage deficiency that occurs with the pure grazing of the traditional farming system. Malnutrition is a major obstacle to the survival, health, growth and reproduction of the animals (Calder and Yaqoob, 2004).

Protein is the most important component of animal diets and plays a vital role in their growth, production and reproduction (Marongiu et al., 2009). Studies indicate that dietary crude protein (CP) deficiency can compromise the immune system (Woodward, 1998; Dasgupta et al., 2005). In addition, following the traditional rearing practice, the mother yak would not return to estrus for approximately one year because she must nurse the calves. Therefore, when a yak gives birth, 1 year of production is generally lost. Long-term malnutrition will necessarily retard normal growth performance for a yak later in life. Early weaning has proven to be an effective means for improving cow productivity (Waterman et al., 2012; Zhou et al., 2012). However, information on the effects of dietary CP supplements on early-weaned yaks is scarce. Moreover, tuberculosis is a serious public health problem and causes significant economic losses in yak production in Tibet (Gao et al., 2012). Vaccination is a low-cost and effective strategy for the prevention and therapeutic reduction of infectious diseases. This strategy has been used effectively to eradicate many bacterial and viral diseases and was highly applicable as a control measure to other infectious diseases including tuberculosis. The standard bovine bacillus Calmette-Guérin (BCG) vaccine is used worldwide against tuberculosis. These situations suggest that there is great potential to improve yak productivity and the immune response of yaks to tuberculosis by supplementing the diet with CP. However, in early-weaned yaks, the underlying mechanisms of the effects of different levels of dietary CP on the immune response to tuberculosis are largely unknown.

Therefore, the objectives of this study were to determine the effects of different levels of dietary CP supplements on the immune response of early-weaned yaks to tuberculosis and to clarify the underlying mechanisms of the immune response to tuberculosis following CP supplements to the diet.

Materials and methods

Experimental procedure

The Sichuan Agricultural University Institutional Animal Care and Use Committee approved all procedures involving animals. The experiments were conducted at Datong (37°11’~37°32’ N, 100°52’~101°54’ E), Qinghai province, China, from September to November 2011. The altitude of the experimental area is approximately 3200 m. During the study, the average temperature was approximately 2.4°C, with 24°C as the highest and -31°C as the lowest temperatures. The rainfall was 463.2 to 636.1 mm, and the evaporation rate was 1054.7 to 1422.3 mm.

Key words: Supplementary feeding; Dietary protein level; Immune response; Yaks.

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All yaks were intramuscularly immunized with BCG prior to the experiment at the age of 30 d. At the age of 90 days, a total of 40 weaned yaks (20 male and 20 female), with an average initial weight of 62.6±0.90 kg, were assigned to 1 of 4 treatments based on body weight and sex. There were 10 (5 male, 5 female) yaks for each treatment. The four dietary treatments included the pasture-only control (CON) and the three pasture plus supplement treatments containing 17, 19 or 21% (LP, MP or HP) CP (Table 1). The animals had 7 days to adapt to the diet and the experimental area, and the experimental period was 60 days. The chemical composition and DMI for each treatment are presented in Table 1. The pasture consisted mainly of lyme grass (Elymus geminatus) for forage. All supplements were offered in pellet form. Yaks received the protein supplements at 16:00~17:00 in the outdoors colony houses (2.0×1.5 m), and the yaks grazed in the pasture at other times.

Sample analyses

The consumption of the supplements was recorded to calculate the average daily feed intake (ADFI) for the experimental period. Yaks were weighed individually at the end of the trial (at the age of 150 days) to calculate the average daily gain (ADG).

Forage samples were collected in three random 1-m² quadrats by clipping whole plants at the ground level on the 30th and 60th days of the experiment. Then, the forage samples were dried for 24 h in a forced-air oven at 65°C, weighed, and ground to pass through a 1-mm screen. Three concentrate samples were collected when the new bags were opened, which were ground through a 1-mm screen. At the conclusion of the study (150 days of age), 10 ml of blood was collected from the jugular vein using heparin sodium coated tubes and centrifuged at 3000×g for 20 min, and the serum was then frozen at -20°C. Blood samples were collected, and the yaks were then slaughtered by exsanguination after electrical stunning. A spleen sample (approximately 0.5 g) was collected, snap-frozen in liquid nitrogen, and then stored at -80°C for total RNA extraction. The intestinal samples were fixed in 4% buffered formalin for 48 h. The processing consisted of serial dehydration, clearing, and impregnation with wax. Tissue sections, 3 m thick (3 cross-sections from each sample), were cut with a microtome and were fixed on slides. A routine staining procedure was performed using hematoxylin and eosin. The slides were examined on an Olympus AX70 microscope (Olympus Corporation, Tokyo, Japan) fitted with a digital video camera (Sony DXC-930P; Sony Corporation, Tokyo, Japan). The images were analyzed using the stereological image software, Cast Image System (Version 2.3.1.3; Visiopharm Albertslund, Horsholm, Denmark). The total of the intact well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section section for each sample. The 10 longest and straightest villi and their associated crypts were measured. The mean values of villous height (VH), crypt depth (CD), and VH/CD within each segment were calculated for statistical analyses. The criterion for villus selection was the presence of an intact lamina propria. VH was measured from the tip of the villus to the villus-crypt junction, and CD was defined as the depth of the invagination between adjacent villi.

The total RNA was extracted from the frozen spleen samples with the TRizol reagent following the manufacturer’s instructions. The RNA was reverse-transcribed using SuperScript III Two-step Reverse Transcript Kit (Invitrogen, Carlsbad, CA, USA).

According to the published sequences of bos taurus beta-actin (β-actin), IL-6, TNF-α, IFN-γ, and NF-AT mRNA at GenBank, the primer sequences for those genes were designed using the Primer premier 5.0 software and are described in detail in Table 2.

A relative quantitative real-time PCR analysis was performed on a Bio-Rad Real-Time PCR detection system (Bio-radcycler version 3.0a; Bio-Rad Laboratories, Hercules, CA, USA). Briefly, 10 µL of 2×SYBR®Premix Ex TaqTM II (TaKaRa Ex Taq HS, dNTP Mixture, Mg²⁺, SYBR® Green I), 0.8 µL of each of primer, and 2.0 µL cDNA were included in a 20 µL PCR. Amplification was conducted with denaturation for 15 min at 95°C, followed by 40 cycles of denaturation for 5 s at 95°C, annealing/elongation for 30 s at 60°C, and a final melting-curve analysis. All target genes were normalized to the endogenous reference gene β-actin by employing an optimized comparative Ct (2-ΔΔCt) value method.

Statistical analyses

All data were analyzed using the MIXED model of the SAS 9.3 software (SAS, 2005). The model used for the analysis was $y = µ + t_i + e_{ij}$, where $y$ is the dependent variable, $µ$ is the population mean for the variable, $t_i$ is the fixed effect of diet treatment (diets=4), and $e_{ij}$ is the.

Table 1. Ingredients and chemical composition of the diets fed to yaks.

| Items               | Control | LP   | MP   | HP   |
|---------------------|---------|------|------|------|
| Corn                | 69.0    | 63.2 | 57.0 |      |
| Protein concentrate  | 30.0    | 35.8 |      | 42.0 |
| Minerals and vitamins| 1.0     | 1.0  | 1.0  | 1.0  |
| Chemical composition|         |      |      |      |
| DM, %               | 90.2    | 88.4 | 87.1 | 86.3 |
| CP, % DM            | 8.2     | 17.2 | 19.1 | 21.2 |
| NDF, % DM           | 49.8    | 15.4 | 17.3 | 18.6 |
| EE, % DM            | 2.3     | 5.2  | 5.45 | 5.45 |
| Ash, % DM           | 1.8     | 2.3  | 2.46 | 2.46 |
| NFC, % DM           | 37.9    | 59.9 | 55.8 | 52.1 |

LP: low protein; MP, medium protein; HP, high protein; DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; EE, ether extract; NFC, non-fibre carbohydrate; 2×SYBR®Premix Ex TaqTM II (TaKaRa Ex Taq HS, dNTP Mixture, Mg²⁺, SYBR® Green I), 0.8 µL of each of primer, and 2.0 µL cDNA were included in a 20 µL PCR. Amplification was conducted with denaturation for 15 min at 95°C, followed by 40 cycles of denaturation for 5 s at 95°C, annealing/elongation for 30 s at 60°C, and a final melting-curve analysis. All target genes were normalized to the endogenous reference gene β-actin by employing an optimized comparative Ct (2-ΔΔCt) value method.

All data were analyzed using the MIXED model of the SAS 9.3 software (SAS, 2005). The model used for the analysis was $y = µ + t_i + e_{ij}$, where $y$ is the dependent variable, $µ$ is the population mean for the variable, $t_i$ is the fixed effect of diet treatment (diets=4), and $e_{ij}$ is the.
random error associated with the observation of growth performance in early-weaned steers, though the diets used in that study increased the incidences of diarrhea in yaks. This may explain why supplementing the diet of yaks with CP improved the growth performance. The supplement with 21% CP over the study period. Very-high-protein diets have been associated with a host of adverse events, including diarrhea, increased calcium excretion from diets high in sulfur-containing amino acids, and increased morbidity (Lemon, 1996). These results indicated that early weaning in yaks was feasible and that growth performance could be improved by CP supplementation; results that may provide a new strategy to improve yak productivity.

The normal structure of small intestine, particularly the VH, CD, and the VH/CD ratio (Yang et al., 2001), is the basic guarantee for the full digestion and absorption of nutrients. The VH and the cell number were significantly correlated, and the villi with the greatest height had the most mature absorptive epithelium cells. CD reflects the rate of cell turnover, and the VH/CD value reflects the comprehensive function of the small intestine (Iwanaga et al., 1993), of which the capacity for digestion and absorption increased with CP supplements. In the present study, the effects of dietary CP supplements were greater on the VH, CD and VH/CD compared with grazing group (P<0.05), and a tendency quadratic effect was found for duodenum and jejunum VH on the different levels of CP supplements (Table 4). These results indicated that all the CP supplemented groups show an improvement of the intestinal morphology, and the 19% CP group was affected the most. Diets containing high CP levels, particularly those with high levels of plant CP that may have strong antigens, may greatly affect villous morphology (Li et al., 1991). Low CP diets reduced VH and CD of the upper small intestine, which was consistent with a report by Nunez et al. (1996). As the CP level decreased to 63 g kg⁻¹diet, VH and CD of the upper small intestine decreased significantly, implying that severe CP deficiency may have reduced villous growth (Nunez et al., 1996). Similar results have been found for pigs fed different levels of dietary CP. Gu and Li (2004) fed piglets diets containing 63, 103, 151, 208 and 14.2 CP g/kg, and they observed higher VH (duodenum, jejunum and distal ileum) and VH and CD (duodenum) in piglets fed diets containing 151 or 103 CP g/kg, respectively. Our data suggested that yaks fed CP supplementation diet show an improvement of the intestinal morphology, and the 19% CP group was affected the most.

Blood urea nitrogen is an end-product of protein catabolism and is often used as an indicator of kidney and liver function and an indication of an animal’s relative hydration status.

### Results and discussion

Protein is the basic component that is used to make all tissues such as muscle, bone, skin, organs and milk. It is important not only for growth but also for repairing and replacing lost cells or tissues. In this experiment, compared with the control group, supplementation with CP increased the BW of yaks at the age of 150 days (P<0.05) and increased the ADG from d 90 to 150 (P<0.05) (Table 3). Yaks fed dietary CP may promote intestinal development and may improve the digestion and absorption of dietary nutrients. Because growth performance data are scarce for early-weaned yaks, no comparisons were possible. However, similar reports were found on cattle. Arthington and Kalmbach (2003) reported that an early weaning group of calves had greater ADG compared with the normal weaning group in the first year. Myers et al. (1999) observed the same improved growth performance in early-weaned steers, though the diets used in that experiment were for finishing beef cattle. Zhang et al. (2014) also observed that supplementing the diet of yaks with CP improved the growth performance. The supplement with 19% CP increased the ADG when compared to the supplements with 17 or 21% CP over the study period (P<0.05). A quadratic effect (P=0.014) was found for ADG on the different levels of CP supplements (Table 3). These results indicated that a 19% CP supplement might be suitable for young yaks. In this study, it also observed that feeding high CP (21% CP) diets increase the incidences of diarrhea in yaks. This may explain why supplementing with 19% CP increased the ADG when compared to the supplements with 21% CP over the study period.

| Gene   | Primer                                                                 | Product size | Accession |
|--------|------------------------------------------------------------------------|--------------|-----------|
| β-actin | F 5'-GATCTGGCACACACCTTTTAC-3' R 5'-GATCTGGGTATCCTTTCTAC-3'             | 115 bp       | AW141970  |
| IL-6   | F 5'-AACATTAGATGTTGGAAGCGACAGCAG-3' R 5'-ACATACCGGCTGCGTACGTGGAC-3'    | 486 bp       | BF040665  |
| TNF-α  | F 5'-TCTCAACGCTCAGAACAAGCC-3' R 5'-TTCTTCGCCGCGTTGTACCTGTC-3'          | 414 bp       | NM_00101266 |
| IFN-γ  | F 5'-GCTTACTCTCCTCTTCTAAGAATAG-3' R 5'-CTCTTCCTCTTCTCTTTTGCTT-3'       | 425 bp       | M29687    |
| NF-AT  | F 5'-CAAGGGGAGACGCGACATCGCGGAG-3' R 5'-ACGGTGCCAGGGCAGTTGGA-3'         | 454 bp       | AW465906  |

IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; NF-AT, nuclear factor of activated T cell transcription factor.

### Table 3. Specific primers used for real-time quantitative polymerase chain reaction.

| Gene     | Primer                                                                 | Product size | Accession |
|----------|------------------------------------------------------------------------|--------------|-----------|
| β-actin  | F 5'-GATCTGGCACACACCTTTTAC-3' R 5'-GATCTGGGTATCCTTTCTAC-3'             | 115 bp       | AW141970  |
| IL-6     | F 5'-AACATTAGATGTTGGAAGCGACAGCAGCAG-3' R 5'-ACATACCGGCTGCGTACGTGGAC-3' | 486 bp       | BF040665  |
| TNF-α    | F 5'-TCTCAACGCTCAGAACAAGCC-3' R 5'-TTCTTCGCCGCGTTGTACCTGTC-3'          | 414 bp       | NM_00101266 |
| IFN-γ    | F 5'-GCTTACTCTCCTCTTCTAAGAATAG-3' R 5'-CTCTTCCTCTTCTCTTTTGCTT-3'       | 425 bp       | M29687    |
| NF-AT    | F 5'-CAAGGGGAGACGCGACATCGCGGAG-3' R 5'-ACGGTGCCAGGGCAGTTGGA-3'         | 454 bp       | AW465906  |

IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; NF-AT, nuclear factor of activated T cell transcription factor.
status (Bossart et al., 2001). A lower concentration of BUN might be due to a decrease in urea synthesis and hydration in the liver and an elevation in the efficiency of dietary CP use. Interestingly, the serum concentration of BUN in all three treatment groups was higher than that in the grazing group (P<0.05) (Table 5). Based on dietary CP contents and DMI, the yaks in the grazing group were 20, 32, 45 and 37 g/d. This calculation will determine the difference between the CP intake from the supplements and the protein retention estimates; in this study, the differences are -20, 53, 48 and 65 g/d. Nitrogen from these proteins could be considered waste and should be detoxified as urea and mainly excreted. BUN is usually highly correlated with urea production in the liver, which is positively correlated with wasted N. Our data suggest that yaks fed different levels of protein concentrate as dietary supplements had lower N use efficiency compared with the grazing group.

Of note, the serum concentration of GLU in yaks of the grazing group was lower than that of the CP supplementation group (P<0.05) (Table 5). Because dietary supplementation with CP may improve the efficiency of GLU and protein absorption, it may promote growth performance. The enzymes AST and ALT provide information on liver, kidney, and other tissue function (Bossart et al., 2001). Increased levels of AST and ALT activity suggest bone injury, growth, or biliary damage. Additionally, ALT is a more specific indicator of liver function, and high levels suggest liver disease, infection, parasitism, or trauma (Lander et al., 2003; Trumble and Castellini, 2002). A notable finding of present study was that the serum activities of ALT and AST in yaks of the grazing group were significantly higher than in the yaks that received the CP supplements (P<0.05) (Table 5). Presumably, supplementation with CP increased metabolic processes and kept these yaks from injury and infection.

The serum antibody level is a useful indicator of humoral immunity. IgG and IgM are key components of the humoral immunity in all mammals and are the major serum immunoglobulins that protect the extracellular compartment against pathogenic viruses and microorganisms (Kong et al., 2007). Additionally, IgG has anti-bacterial and anti-toxin effects (Li et al., 2007). The yaks in the grazing group had significantly less elevation in the serum concentrations of IgG and IgM than did the dietary CP supplementation group (P<0.05) (Table 6). Having adequate levels of proteins and amino acids in the diet is essential for maintaining cellular function. Because the characteristic element of protein is nitrogen, which constitutes 16% of protein weight, nitrogen metabolism is often considered to be synonymous with protein metabolism in the body. Dietary CP supplementation was essential to maintain cellular function, which was beneficial for the BCG vaccine because of the increased production of antibodies by B-lymphocytes. Our data suggest that dietary CP supplements could benefit the BCG vaccine by improving humoral immunity with increasing serum levels of IgG and IgM in yaks.

The present results showed that the serum

### Table 4. Effects of different levels of protein supplements on intestinal morphological criteria of yaks.

| Intestines | Control | Supplement level | SEM | Linear | Quadratic |
|------------|---------|------------------|-----|--------|-----------|
| **Duodenum** | | | | | |
| CD, μm | 157.2* | 187.1bc | | 264.9 | 224.5b | 6.53 | 0.177 | 0.008 |
| VH, μm | 345.5* | 413.5bc | 505.0 | 465.6bc | 12.31 | 0.319 | 0.091 |
| VH/CD | 2.21* | 2.20 | 1.94 | 2.13 | 0.069 | 0.720 | 0.470 |
| **Jejunum** | | | | | |
| CD, μm | 180.0* | 208.1c | 340.5 | 301.8b | 5.36 | 0.064 | <0.001 |
| VH, μm | 427.9* | 450.6a | 564.6a | 544.4a | 14.89 | 0.095 | 0.062 |
| VH/CD | 2.38* | 2.17c | 1.65b | 1.81c | 0.043 | 0.033 | 0.002 |
| **Ileum** | | | | | |
| CD, μm | 182.2* | 224.6c | 387.6 | 333.3b | 6.44 | 0.010 | <0.001 |
| VH, μm | 399.3* | 497.3bc | 589.1b | 520.4b | 9.21 | 0.337 | 0.016 |
| VH/CD | 2.20* | 2.22b | 1.52b | 1.59b | 0.046 | 0.002 | <0.001 |

LP: low protein; MP: medium protein; HP: high protein; CD: crypt depth; VH: villous height; VH/CD: villous height-crypt depth ratio. *Significantly different from all protein supplement groups (P<0.05).

### Table 5. Effects of different levels of protein supplements on serum biochemical parameters of yaks.

| Serum biochemical parameters | Control | Supplement level | SEM | Linear | Quadratic |
|-------------------------------|---------|------------------|-----|--------|-----------|
| BUN, mmol/L | 2.13* | 3.38b | 2.83b | 2.96b | 0.062 | 0.022 | 0.006 |
| GLU, mmol/L | 3.27* | 4.69b | 4.15b | 4.17b | 0.096 | 0.044 | 0.059 |
| TP, g/L | 86.56 | 88.21 | 90.04 | 88.02 | 0.614 | 0.098 | 0.389 |
| ALB, g/L | 41.28* | 37.36 | 36.10 | 33.67 | 0.798 | 0.104 | 0.264 |
| ALT, μL | 16.20* | 13.60 | 12.57 | 13.70 | 0.437 | 0.939 | 0.671 |
| AST, μL | 51.70* | 46.70 | 45.70 | 42.90 | 0.863 | 0.115 | 0.269 |

LP: low protein; MP: medium protein; HP: high protein; BUN, blood urea nitrogen; GLU, glucose; TP, total protein; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase. *Significantly different from all protein supplement groups (P<0.05). **Values within a row with different letters are significantly different (P<0.05).
levels of TNF-α, IFN-γ and IL-2 in all three treatment groups were higher than those of the grazing group (P<0.05), and a tendency quadratic effect was found for IFN-γ on the different levels of CP supplements (Table 6). Although the underlying mechanisms were unclear, supplementing the diet with CP increased animal growth, which is beneficial for the BCG vaccine because supplements increased the serum levels of IL-1, IL-2, TNF-α and IFN-γ. Cytokines play a central role in the cell-mediated immune response, and they participate in the maintenance of tissue integrity (Piel et al., 2004). During the inflammation process, IL-1 is produced by polymorphonuclear leukocytes that have numerous functions, including the stimulation of the synthesis and release of acute phase reactants, the augmentation of T cell and B cell activation, and the induction of other regulatory cytokines such as IL-6 and IL-8. Interleukin-2 is a broad-spectrum promoter of immune activity that can induce the differentiation of T cells and B cells, promote the function of the NK cell, and release antitumor and antiviral interferon. TNF-α and IFN-γ, acting as inflammatory mediators, are induced in response to infection and exert a number of different effects in various cells; thus, they initiate and regulate the immune response (Bemelmans et al., 1996). Our data suggest that yaks fed dietary CP could benefit from improvement in the effectiveness of the BCG vaccine due to the enhanced humoral immunity with increased serum levels of IL-1, IL-2, TNF-α and IFN-γ in yaks fed CP supplements.

Our results showed that IL-6, TNF-α, IFN-γ and NF-AT gene expression increased significantly (P<0.05) when the diet was supplemented with CP. IL-6, IFN-γ and NF-AT gene expression showed quadratic increases as the dietary CP increased from 17 to 21% (Table 7). Nuclear factor of activated T cell transcription factor is a family of transcription factors critical in regulating early gene transcription in response to T cell receptor-mediated signals in lymphocytes (Ranger et al., 2000). A high expression of NF-AT mRNA results in a high expression of the cytokines, such as IL-6 (Liu et al., 2004), TNF-α (Tsytyskova and Goldfeld, 2000) and IFN-γ (Sweetser et al., 1998). Similar to all cells of the body, immune cells need nutrients to function, multiply and, when the immune system detects a pathogen, trigger a systemic alarm. Appropriate supplementation of dietary CP increased animal growth, which likely benefited the BCG vaccine by up-regulating the mRNA expression of immunefunction related factors through a complicated signal pathway and thereby improving the immune status of yaks.

### Conclusions

In conclusion, dietary CP supplementation improved the growth performance and intestinal development of early-weaned yaks. Thus, the CP supplements were beneficial and enhanced the humoral immunity response of yaks, and the 19% CP group was affected the most.

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### Table 6. Effects of different levels of protein supplements on serum concentrations of immunoglobulins and cytokines in yaks.

| Immunoglobulins and cytokines | Control | Supplement level | SEM | P     |
|------------------------------|---------|------------------|-----|-------|
|                             | LP      | MP               | HP  | Linear | Quadratic |
| IgG, g/L                    | 2.53*   | 3.28             | 3.50| 3.43   | 0.118     | 0.662 | 0.807 |
| IgM, g/L                    | 0.25*   | 0.34             | 0.41| 0.36   | 0.012     | 0.463 | 0.111 |
| IL-1, pg/mL                 | 18.32*  | 27.40            | 25.50| 24.20  | 0.065     | 0.080 | 0.219 |
| IL-2, ng/mL                 | 3.84*   | 3.45             | 3.92| 3.41   | 0.094     | 0.685 | 0.152 |
| TNF-α, ng/mL                | 5.64*   | 4.35             | 4.70| 4.38   | 0.089     | 0.902 | 0.384 |
| IFN-γ, U/mL                 | 79.7*   | 82.8             | 87.4^b| 88.8^b| 0.873     | 0.022 | 0.053 |

LP, low protein; MP, medium protein; HP, high protein; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-1, interleukin-1; IL-2, interleukin-2; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ.

### Table 7. Effects of different levels of protein supplements on relative cytokine gene expression in the spleen of yaks.

| Gene expression | Control | Supplement level | SEM | P     |
|-----------------|---------|------------------|-----|-------|
|                 | LP      | MP               | HP  | Linear | Quadratic |
| NF-AT mRNA      | 1.00*   | 1.19^b           | 1.47^a| 1.30^a| 0.031     | 0.268 | 0.004 |
| IL-6 mRNA       | 1.00*   | 1.24^b           | 1.49^a| 1.19^a| 0.037     | 0.717 | 0.027 |
| TNF-α mRNA      | 1.00*   | 1.25             | 1.46| 1.33   | 0.035     | 0.490 | 0.181 |
| IFN-γ mRNA      | 1.00*   | 1.25^b           | 1.59^a| 1.38^a| 0.036     | 0.205 | 0.001 |

LP, low protein; MP, medium protein; HP, high protein; NF-AT, nuclear factor of activated T cell transcription factor; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ.*Significantly different from all protein supplement groups (P<0.05). ^Values within a row with different letters are significantly different (P<0.05).
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