Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
29 (6): 649-657, 2023
DOI: 10.9775/kvfd.2023.30159

RESEARCH ARTICLE

Effects of Dietary Protein on Milk Yield and Colostrum Whey Protein Composition of Tibetan Sheep in Modern Intensive-fed Pattern

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How to cite this article?

Ren H, Chen Q, Zhang Y, Zhang H, Jia J: Effects of dietary protein on milk yield and colostrum whey protein composition of Tibetan sheep in modern intensive-fed pattern. *Kafkas Univ Vet Fak Derg,* 29 (6): 649-657, 2023.
DOI: 10.9775/kvfd.2023.30159

Article ID: KVFD-2023-30159
Received: 29.06.2023
Accepted: 08.10.2023
Published Online: 25.10.2023

ABSTRACT

Colostrum protein, an essential source of dietary nutrients, could improve new-born animals' immunity, and play a vital role in mammals' early development. In order to explore the milk yield and colostrum whey protein composition of Tibetan sheep, 120 Tibetan sheep were arbitrarily separated into categories, namely treatment groups (A, B, C) and control group (D). Compositional and functional differences in milk yield and colostrum whey protein composition among different dietary proteins were compared using proteomics methods. The results showed that sheep with 14% protein level diet group (group B) had the least bodyweight loss and higher milk yield during lactation compared to the other groups. Fifty differentially expressed proteins (DEPs) were recognized using iTRAQ, these DEPs were analyzed based on cluster, GO, KEGG and PPIs analysis. GO-BP involved were Protein transmembrane transport, Protein regulation metabolic process, Biological regulation, Regulation of biological process, and Response to stimulus. Meantime, DEPs participated in many KEGG pathways, including Fatty acid metabolism, Glycerophospholipid metabolism, Protein digestion and absorption, Ras signaling pathway and Cell adhesion molecules. The treatment groups showed increase in the abundance of regulation metabolic process (especially protein metabolism and fatty acid metabolism), along with decrease in stress reaction process. Lactoferrin, Alpha-S2-casein, Superoxide dismutase [Cu-Zn], Alpha-s1-casein, Alpha globin and Lactoperoxidase appeared in the center of the PPi network intersection. Interestingly, 14% protein group (group B) had exhibited the greatest variability between biological relevance in milk composition and function, these results could increase the understanding of different dietary protein on colostrum whey protein composition of Tibetan sheep, which could provide important information and potential directions for the infant milk powder and functional food industries.

Keywords: Tibetan sheep, Dietary protein, Milk yield, Colostrum whey protein, Proteomics

INTRODUCTION

Tibetan sheep grazed grassland all year round with traditional grazing management, and the herbage and nutrients were often insufficient to maintain the normal physiological function in cold season, which would result low ewe lactation and high lamb mortality [1,2]. The study showed that dietary proteins have many nutritional and biological functions, and dietary protein bioavailability directly affects animal production performance [3,4]. There are positive effects of dietary protein on sheep productivity and reproductive performance, which could reduce body weight-loss and feeding costs, and increase economic efficiency [5]. Therefore, there is a big potential to improve sheep lactation performance and milk composition through developing protein-diet supplementary system during cold season.

Protein is the basic material of mammal living activities; dietary protein level is the limiting nutrient element that affects the sheep milk yield and lactoprotein [6]. When varying the quantitative of protein-diet supplementation in the diet, milk compositions in ruminants may fluctuate due to a change in nutritional intake [7]. Many studies reviewed that the protein-diet supplementation in the diet exhibited prominent effect on the sheep milk yield, especially in the early stages of lactation [8]. Moreover, the appropriate dietary crude protein level in the later period of pregnancy and lactation could improve milk yield and lactoprotein content [9]. Once there is a lack

of dietary protein, the dry matter intake of sheep could decrease, which results in a decrease in milk yield. Meanwhile, the high dietary protein, which exceeded the needs of maintenance and lactation, would also have a negative impact in milk yield and milk composition [10,11]. Therefore, the regulation of dietary protein nutrition was an important link in the production of sheep during lactation.

The conversion of protein in feed into available nutrients is very important for mammalian production traits, and the nitrogen deposition of high protein diet is significantly greater than that in low protein diet, which led to increase of protein deposition, promote the growth and development, and improve animal final body weight and ADG with the increase of dietary protein [12,13]. However, protein utilization rate in ruminants is usually lower than that of monogastric animals. A large proportion of dietary protein is not effectively utilized by ruminants, and the unutilized nitrogen is expelled through metabolic process, which could result in environmental nitrogen pollution [14]. In the present study, we measured and analyzed Tibetan sheep milk yield and colostrum composition with different protein-diet supplementary, the change rule and biological significance of dietary protein level on milk proteome difference, which provided basic data for Tibetan sheep feeding and development of feed products.

Material and Methods

Ethics Statement

Animal experiment was approved by the Institutional Animal Care and Use Committee (State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University) (QHDX-19-10-07-06).

Study Site

The study was done at Tibetan Autonomous Prefecture of Hainan, Qinghai Province of China, situated at south of Qinghai-Tibetan Plateau. This area is over 3200 m above the sea level and has a dry cold climate. The experimental Tibetan sheep were feed in standardization shed, and processed according to experimental design [15].

Animals and Diets

One hundred-twenty Tibetan ewes (single lamb trait) were selected, which were the same body weight (43.39±2.20 kg) and age (3-4 years old, 2nd birth orders). Before this study, individuals grazed only natural pasture and were not offered supplements. The experiment lasted 75 days from December, 2017 to March, 2018 (15 days before parturition was an adjustment period and the following 60 days was for sample collection period). Different levels of dietary protein supplements were provided for the Tibetan sheep, which were grazed on the natural grassland until the end of the experiment. All ewes were allotted randomly into four groups. Group A, B and C were fed 12%, 14% and 16% dietary protein, respectively (Treatment Group, A, B and C). Group D was fed no supplement (Control Group, CON). The dietary formula and nutritional components were showed in *Table 1*. The experimental diets were formulated according to the nutrient requirements of an ewe weighing 40 kg (NRC 2007 and Standards for Feeding Sheep of China NYT816-2004). Diets were composed of the most popular feed resources in Qinghai-Tibetan plateau as feeding standard, 1.8 kg (dry matter) per individual and day of a total mixed ration. The nutrient composition of mixed-feed was analyzed or tested by'Feed Analysis and Quality Test Technology'. [16].

Measurement of Samples

The ewes were weighed at day 1 and 60 of lactation period by using platform scale before feeding in the morning.

The ewes were milked twice daily at 08:00 and 18:00 via a milking machine, daily individual milk yield was recorded and then kept at the dairy laboratory of Key of

Table 1. *Ingredients and chemical composition of the experimental diets (DM)*

Items	**Proportion (%)**			
Ingredients	**A**	**B**	**C**	**D**
Corn	52.00	47.00	41.50	
Soybean meal	2.00	6.50	13.00	
Cottonseed meal	3.00	5.00	5.00	
Bran	1.50	1.00	0.50	
Rapeseed meal	7.00	5.80	5.00	
Oaten Hay	10.00	10.00	10.00	
Ensiling Corn	20.00	20.00	20.00	
Limestone	0.50	0.70	1.00	
Premix[1]	4.00	4.00	4.00	
Total	100.00	100.00	100.00	
Nutrient Content				
DM	72.47	72.48	72.40	94.51
CP	12.23	14.12	16.13	5.33
DE (MJ/Kg) [2]	12.13	12.11	12.09	4.12
EE	2.93	2.81	2.71	2.69
ADF	12.83	13.26	13.54	42.17
NDF	23.53	23.79	23.79	60.23
Ca	0.35	0.43	0.55	3.77
P	0.34	0.36	0.37	0.03

[1] *The premix provided the following per kg of dietary: Vit. A: 12.000 IU, Vit. D: 2000 IU, Vit. E: 30 IU, Cu: 12 mg, Fe: 64 mg, Mn: 56 mg, Zn: 60 mg, I: 1.2 mg, Se: 0.4 mg, Co: 0.4 mg*
[2] *Digestible Energy (DE) was the calculated value, and others were the measured values*

Laboratory of Plateau Ecology and Agriculture (Qinghai University).

Individual milk samples from 08:00 and 18:00 were collected at lactation period (1d, 3d, 5d, 10d, 15d, 20d, 25d, 28d, 30d, 33d, 35d, 40d, 45d, 50d, 55d and 60d) and collected 5 repeats of each milk sample. All the milk samples were carried to the laboratory and processed in order to assess the normal composition of nutrition, such as fat, skim solids, lactose, protein and milk density, by using milk composition measuring instrument (MT-100, China). The colostrum (1-10 day postpartum) samples were centrifuged at 4000 r/min for 20 min at 4ºC. Fat fractions of the milk samples were carefully removed. The skim milk samples were centrifuged at 12000 r/min for 60 min at 4ºC to detect the protein concentration by Bradford method.

The whey was collected to determine the milk proteome difference of the 4 groups by iTRAQ technology (Isobaric Tags for Relative and Absolute Quantification), and whey proteomic analysis was outsourced to Shanghai Majorbio Bio-pharm Technology (SMBPT) Co., Ltd (China, Shanghai). Protein digestion was performed according to the filter-aided sample preparation (FASP) procedure, described by Wisniewski, and the resulting peptide mixture was labeled using the 4-plex iTRAQ reagent (AB SCIEX, Foster City, CA, USA), according to the manufacturer's instructions. A total of 30-μg peptide mixture was labeled with iTRAQ reagents according to the manufacturer instructions (Applied Biosystems, USA). Group 1 samples were labeled with reagent 114, group 2 with reagent 115, group 3 with reagent 116, and group 4 with reagent 117. The labeling reaction was performed by 1-h incubation at room temperature. The iTRAQ-labeled peptides were fractionated by SCX chromatography using the AKTA Purifier system (GE Healthcare, Fairfield, CT, USA). Experiments were performed on a Q Exactive mass spectrometer that was coupled to an Easy nLC (ThermoFisher Scientific, Waltham, MA, USA). The MS/MSspectra were searched using the MASCOT search engine (Matrix Science, London, UK; version 2.2) embedded into Proteome Discoverer 1.4 (Thermo Electron, San Jose, CA, USA) against the uniport database (91,245 sequences, download at 20171210) and decoy database. Differentially expressed proteins (DEPs) were based on standards of a 1.2-fold change in abundance (ratio ≥1.20 or ≤0.833) and $P<0.05$.

Statistical Analysis

Bioinformatics was analyzed via R language toolkit [17]. Functional annotation and classification of all identified proteins were determined using the Blast2GO and InterProScan program against the Uniprot database (uniprotsheep 515149.fasta). Pathway analyses were extracted using the search pathway tool of the KEGG mapper platform *(http://www.genome.jp/kegg/)* and BLAST program. Pathway enrichment statistics were conducted by the Fisher's exact test, and the pathways with a corrected $P<0.05$ were defined as the most significant pathways. The STRING program *(http://string-db.org/)* for the retrieval of interacting genes/proteins database for the prediction of the physical and functional interactions was used to analyze the PPIs. The graphical visualization and analysis of the interaction network were performed in Cytoscape software.

The data were expressed as mean ± standard deviation (SD). Duncan's post hoc test was used to determine any significant differences among 4 groups. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.1$.

Results

Body Weight Changes During Lactation Period

The initial body weights, final weights and live weight gain of Tibetan ewes during lactation period in different dietary protein level were presented in *Table 2*. The data indicated that the live weight gain was positive increase in treatment groups and a decrease in control group during lactation period. The final weights can be significantly increased in treatment groups than in control group ($P<0.05$). As far as treatment groups were concerned, the effect of group B and C were significantly better than group A in live weight gain ($P<0.05$). There was no significant difference between group B and group C ($P>0.05$), while the 14% protein group (group B) had more obvious effect in live weight gain.

Milk Yield and Lactation Regularity

The results of milk yield and lactation regularity were summarized in *Fig. 1* and *Table 3*. The present results showed that administered dietary protein levels significantly affect milk yield. Maximum milk yield (30d) and average milk yield of various stages (10d, 0-60d, 0-10d, 10-30d and 30-60d) of Tibetan ewes during lactation period were significantly higher in treatment groups than those of control group ($P<0.05$). The milk yield of 14% protein level diet was significantly higher than 12% and 16% protein level diet during whole lactation period ($P<0.05$). All of the groups had same lactation regularity, the milk yield showed a downward trend from 0 to 10 days, then showed a rising trend from 10d to 30d, and milk yield reached highest point on the 30d, and then showed a downward trend from 30 to 60 days. And 14% protein level diet group (group B) was greater milk yield than other groups during the whole lactation period ($P<0.05$). The results of milk composition analyses at the 0-10d were summarized in *Table 4*. Compared to control

Table 2. *The body weight changes of Tibetan ewes in different dietary protein levels*

Groups	N	Initial Weight (kg)	Final Weight (kg)	Live Weight Gain (kg)
A	30	43.53±0.96	49.88±0.85[b]	6.35±0.45[b]
B	30	43.62±1.15	54.10±1.07[a]	10.48±0.69[a]
C	30	44.39±1.20	53.40±1.11[a]	9.01±1.03[a]
D	30	44.18±1.08	42.86±1.94[c]	-1.32±0.57[c]

[1] *Group A was fed the 12% dietary protein level group, Group B was fed the 14% dietary protein level group, Group C was fed the 16% dietary protein level group, Group D was the control group*
[2] *The initial weight was measured on December 31, 2017, the final weight was measured on March 1, 2018*

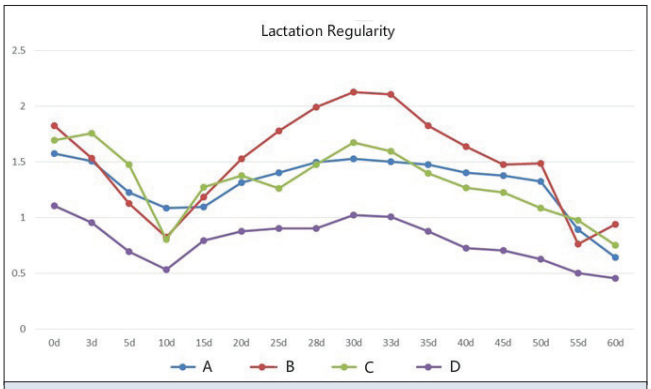

Fig 1. The lactation regularity of Tibetan ewes in different dietary protein levels

Table 3. *The milk yield of Tibetan ewes in different dietary protein levels (kg)*

Groups	Average	10d	30d	0-10d	10-30d	30-60d
A	1.302[b]	1.202[a]	1.525[b]	1.348[a]	1.367[b]	1.23[b]
B	1.509[a]	0.772[b]	2.125[a]	1.328[a]	1.72[a]	1.461[a]
C	1.318[b]	0.769[b]	1.675[b]	1.433[a]	1.412[b]	1.184[b]
D	0.793[c]	0.502[c]	1.025[c]	0.823[b]	0.901[c]	0.699[c]

[1] *Group A was fed the 12% dietary protein level group, Group B was fed the 14% dietary protein level group, Group C was fed the 16% dietary protein level group, Group D was the control group*

group, the increases in lactose of treatment groups were significantly (P<0.05). No significant difference existed in the proportions of milk fat, skim solids, protein and milk density at any time point (P>0.05).

Table 4. *The 0-10d milk composition of Tibetan ewes in different dietary protein levels (%)*

Groups	Fat	Skim Solids	Lactose	Protein	Density
A	4.414	11.212	6.737[a]	4.762	33.842
B	4.466	11.606	6.626[a]	4.974	33.773
C	4.382	11.131	6.483a	4.922	33.847
D	4.406	11.396	5.039[b]	4.836	33.258

[1] *Group A was fed the 12% dietary protein level group, Group B was fed the 14% dietary protein level group, Group C was fed the 16% dietary protein level group, Group D was the control group*

Changes in Proteome Profiles During Different Dietary Protein Levels

Three hundred and forty six non-redundant proteins with *Ovis aries* database were successfully identified via Mascot and iTRAQ method. Then we applied a manual thresholding approach and a probabilistic prediction algorithm, yielding 310 high-confidence candidates. A total of 50 differentially expressed proteins in different groups using 1.2-fold and a P-value <0.05 of differentially expressed protein were identified from the 310 proteins. The expression levels of 37 proteins were up-regulated in samples in treatment groups and 13 were down-regulated (*Fig. 2*). Interestingly, 14% protein group showed the highest up-regulated/down-regulated trends in up-regulated proteins and down-regulated proteins.

Sheep milk differentially expression proteins were directly subjected to hierarchical clustering by Cluster 3.0 software,

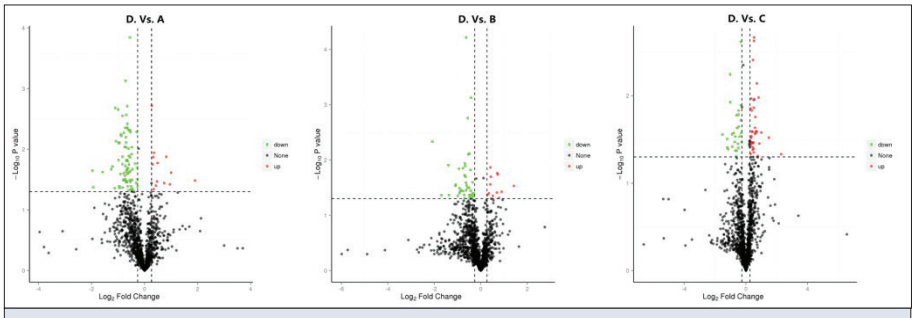

Fig 2. Differentially expressed proteins, including 3 replicates with Tibetan sheep colostrum whey during treatment groups (A, B, C) and control group (D). The image presents the relative abundance of proteins using different colors, where deeper red represents higher intensity and blue represents lower intensity

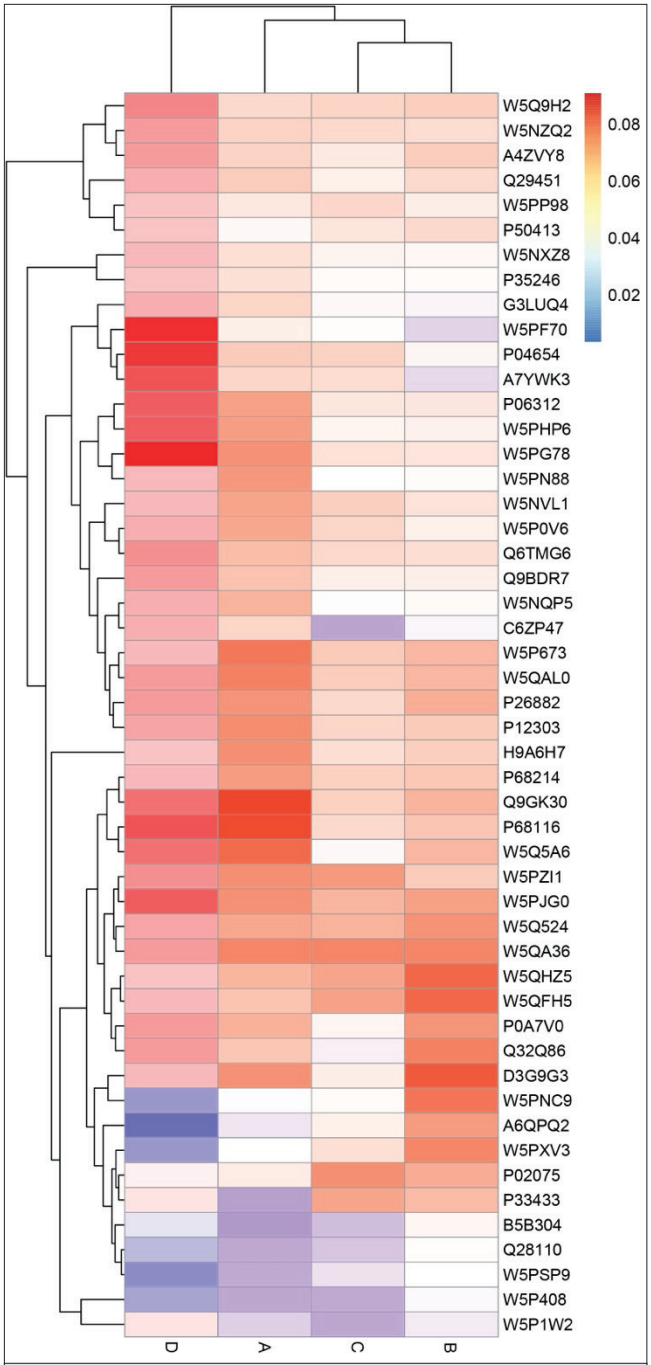

Fig 3. Differentially Expressed Proteins (DEPs) Cluster Evaluation. Down was the DEPs of treatment group were less than control group; Up was the DEPs of treatment group were more than control group; None was the DEPs of treatment group were no significant differences than control group

which yielded a pattern consisting of two major sample clusters. The sheep milk of treatment groups shared similar proteomic patterns and 14% protein dietary level and 16% protein dietary level milk comprised one sub cluster and 12% protein dietary level milk joined this group to constitute another sub cluster. The sheep milk of control group was comprised of another sub cluster *(Fig. 3)*. Although the sheep milk of treatment groups was the same differentially expression proteins patterns, hierarchical clustering analysis revealed differences in sheep milk of different protein level dietary. These differentially expression proteins are clearly presented in the hierarchical clustering map via view tree software.

We further performed biological function process and signaling pathways to investigate the function of these differentially expression proteins. BLAST2GO software and KAAS database were used to participate the 30 biological function process (GO-BP) and 20 signaling pathway of 50 differentially expression proteins *(Fig. 4, Fig. 5)*. The top 5 biological process categories were: Protein transmembrane transport (21.0%), Protein regulation metabolic process (15.6%), Biological regulation (9.1%), Regulation of biological process (6.9%), and Response to stimulus (6.4%). And top 5 pathways categories were: Fatty acid metabolism (P=0.06), Glycerophospholipid metabolism (P=0.10), Protein digestion and absorption (P=0.13), Ras signaling pathway (P=0.14) and Cell adhesion molecules (P=0.18). The treatment groups showed increase in the abundance of regulation metabolic process (especially protein metabolism and fatty acid metabolism), along with decrease in stress reaction process.

The protein-protein interaction network was produced for the 50 differentially expressed proteins via the database at www.string-db.org *(Fig. 6)*. As expected, the target proteins constituted a complex and strong PPi network, and those results of this analysis identified that Lactoferrin, Alpha-S2-casein, Superoxide dismutase [Cu-Zn], Alpha-s1-casein, Alpha globin and Lactoperoxidase appeared in the center of the PPi network intersection indicating their important role in the protein interactions. Hence, DEPs could be vital for function and physiological operation in addition to the protein composition of the Tibetan sheep colostrum whey. Interestingly, to provide further insights into the biological processes identified by this approach, we took Fishers' test (Significance A/B Test) for target proteins, those sixproteins from our results were not only at the center of the functional network intersection, but also exhibited the greatest variability between biological relevance in milk composition.

To elucidate the correspondence between the transcript level of mRNA and abundance of protein species, transcriptional analysis of 6 differentially expression protein were performed by qPCR *(Fig. 7)*. The transcript levels of four genes displayed the same trend with the abundance of the corresponding protein species, such as Lactoferrin, Superoxide dismutase [Cu-Zn], Alpha globin and Lactoperoxidase. In contrast, the expression level of two genes (Alpha-S2-casein and Alpha-s1-casein) showed the opposite trend with the abundance of their corresponding protein species. The discrepancy between the transcription level of the two genes and the

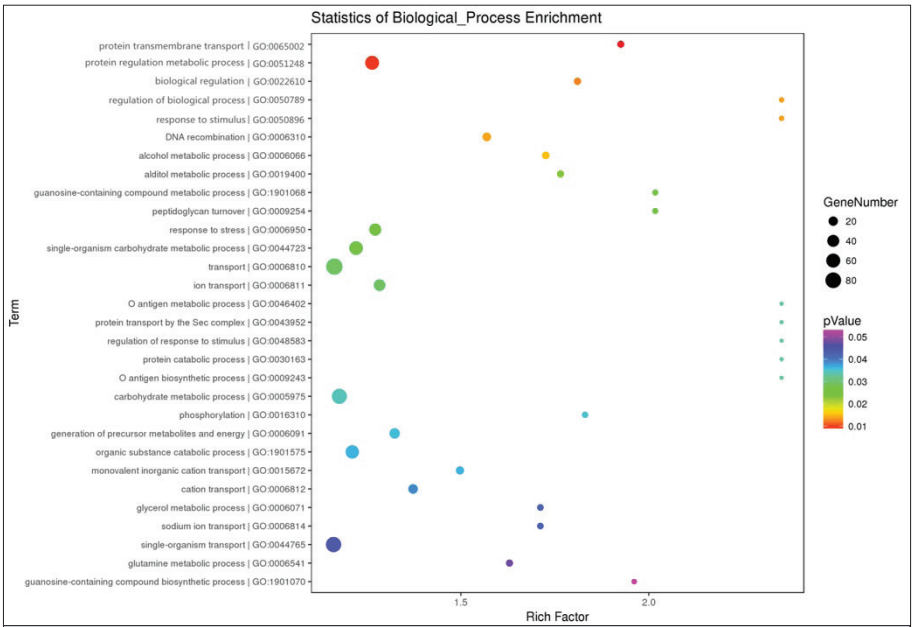

Fig 4. Differentially Expressed Proteins (DEPs) GO-BP Enrichment Evaluation

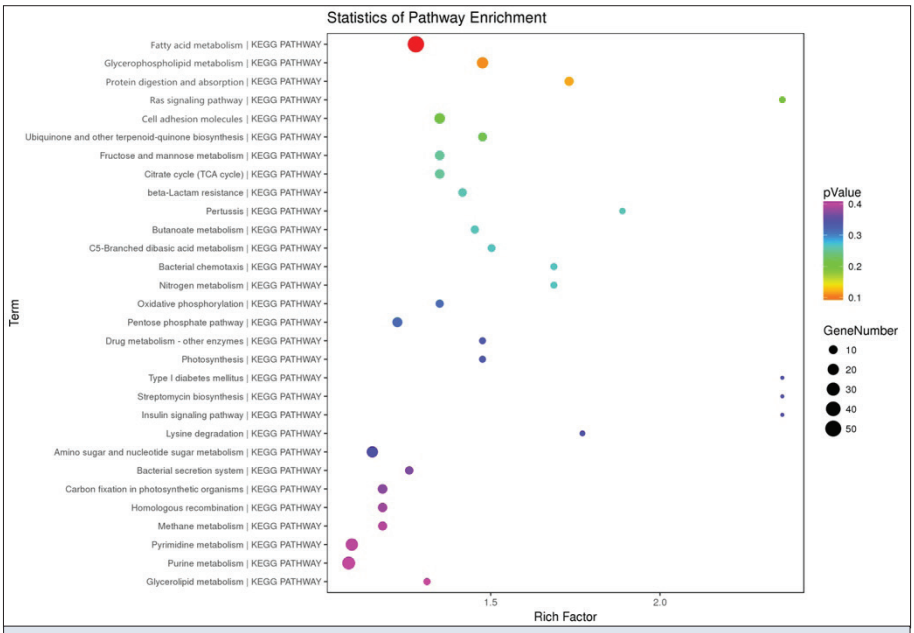

Fig 5. Differentially Expressed Proteins (DEPs) KEGG Enrichment Evaluation

abundance of the corresponding protein species probably resulted from various posttranslational modifications under different dietary protein level stress, such as protein phosphorylation and glycosylation.

Discussion

Proteins are substances for basis of life and specific practitioners of life activities [18]. Dietary protein was the limiting nutrient element that affects mammal milk yield and lactoprotein, and milk protein intake was the key factor for the survival of newborn lambs [19]. The major challenge for ruminant milk researchers was the complexity of the milk composition response to dietary composition [20]. Some researchers showed that reducing dietary protein content in a certain range could improve milk nitrogen efficiency and reduce nitrogen excretion through feces and urine [21]. Although milk composition has been extensively studied in the fields of proteomics, epigenetics, transcriptomics, and molecular biology, the mechanisms by which dietary protein composition affects milk composition still need to be further elucidated.

Colostrum intake was the key factor for the survival of newborn mammals, which contained many special

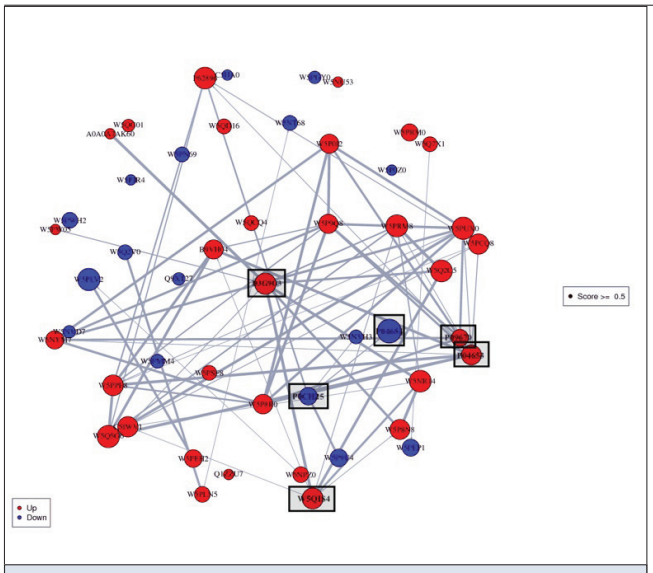

Fig 6. Differentially Expressed Proteins (DEPs) PPIs Evaluation. The marked zone was enriched DEPs, the red points were up-regulated DEPs. and the blue points were down-regulated DEPs

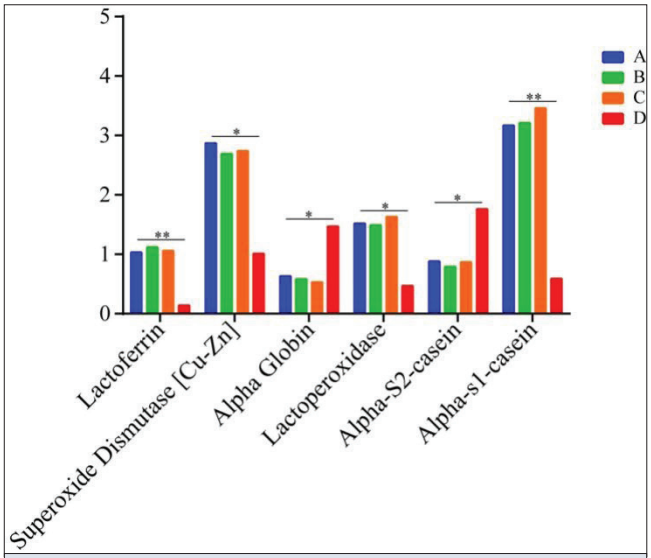

Fig 7. Effects of different dietary protein levels on the differentially expressed proteins coding genes in Tibetan sheep colostrum whey during treatment groups (A, B, C) and control group (D)

function proteins to promote development of gastrointestinal tract and improve immunity [22]. The milk nutrients could be obtained in the biosynthesis of mammary gland epithelial cells by using the raw materials in the blood [23,24]. Therefore, the various components of milk derive from the blood, and the nutrients in the blood were provided by the feed. In present study, we screened 50 differentially expressed proteins that demonstrated differential expression in sheep milk with different protein level dietary using iTRAQ technology, we found that 6 proteins (Lactoferrin, αS1-casein, Superoxide dismutase [Cu-Zn], αS2-casein, Alpha globin and Lactoperoxidase) had exhibited the greatest variability between biological relevance in milk composition and warrant further study. Previous studies have demonstrated that dietary protein levels determine αS1-casein protein synthesis and are particularly significant in high-protein diets [25]. This coincides with our findings.

NRC estimated the effect of diet protein on milk yield based on 393 data from 82 experiments showed the mammal can make better use of protein in low protein diet condition, but high protein diet had little effect on milk performance, increased nitrogen excretion, and resulted in low protein utilization efficiency [14]. The six candidate target proteins played an important role in improving Tibetan sheep milk yield and colostrum whey protein composition in our experiment. Superoxide dismutase [Cu-Zn], like Alpha globin, was an active substance derived, which could eliminate harmful substances produced in the process of metabolism. It could regulate the body immunity, which is closely related to the early pregnancy and immune tolerance of mammals. The intake of colostrum could not only increase the ewes' content of immune protein, but also increase the content of lambs' non-immune protein [26]. Lactoferrin (IF) was a kind of non heme iron binding glycoprotein with high biological activity, which was rich in mammalian colostrum [27]. In mammalian body, LF had the functions of balancing the iron element promoting the growth of intestinal beneficial bacteria, enhancing the immunity, broad-spectrum antibacterial and antiviral, regulating body metabolism, and acting as the activator of cell growth promoting factor. Meanwhile, it could also be used as a transcription activator or trans-activator to bind cell receptor, and participate in mitogen activated protein kinase/extracellular signal regulated kinase interference and nuclear factor NK-κB immune response pathway to promote the maturation of T lymphocytes [28], activate the activity of natural killer cells, release IL-1 and IL-2 to play the role of immune regulation [29]. Casein was the main protein in sheep milk, which had high nutritional value. αS1-casein and αS2-casein were the main components of casein. αS1-casein and αS2-casein were the highly phosphorylated proteins [30]. After enzymatic hydrolysis and phosphorylation, they could combine with calcium, magnesium, iron, zinc and copper to form soluble phosphopeptides, thus promoting the absorption of metal ions by the body [31]. The phosphorylated casein in sheep milk could not only resist the hydrolysis of various enzymes in the digestive tract, but also form soluble substances with calcium to prevent calcium from forming calcium phosphate precipitation [32]. Meanwhile, it could effectively prolong the retention time of calcium in the body, promote the absorption of mineral elements in the intestine, promote in vitro fertilization of animals, enhance immunity and induce apoptosis. lactoglobulin was a member of the lipid transporter family [33]. The

milk specific protein synthesized by mammary epithelial cells was a high-quality protein with the best proportion of amino acids and high content of branched chain amino acids [34]. For newborn lambs, lactoglobulin had the function similar to immunoglobulin, and had the physiological activities such as antibacterial, antiviral, antioxidant, etc. for example, when vitamin E was deficient in the body, and the protein could improve the content of reduced glutathione in the liver, thus enhancing the antioxidant capacity of cell membrane [35]. In our study, 14% protein level supplementary feed could optimize sheep milk Superoxide dismutase [Cu-Zn], Alpha globin, LF, αS2-casein, αS1-casein and Lactoglobulin content, which would be helpful for lamb's growth and development. The casein result of qPCR was contrary to iTRAQ, which might be caused by the inconsistency of mRNA and protein expression due to the post transcriptional phosphorylation of α-casein. Previous studies have demonstrated that α-caseind expression is regulated by transcription factors [36].

Availability of Data and Materials

The authors declare that data supporting the study findings are also available to the corresponding author (J. Jia).

Acknowledgments

The authors are grateful to all the participants who took part in this study.

Funding Support

This work was supported by Natural Science Foundation of Gansu (No. 22CX2NA005) and Natural Science Foundation of Shandong Province (ZR2023MC164).

Conflicts of Interest

The authors declare no conflict of interest.

Ethics Statement

Animal experiment was approved by the Institutional Animal Care and Use Committee (State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University) (QHDX-19-10-07-06).

Authors' Contributions

Hao REN and Qian CHEN: the hypothesis of this study; Hao REN and Yingying ZHANG: work management, article writing; Qian CHEN and Huaixia ZHANG: experimental procedure follow-up, statistical analysis; Qian CHEN and Yingying ZHANG: literature review, review of results; Jianlei JIA: final decision, funding support.

References

1. Wei CH, Wang HH, Liu G, Zhao FP, Kijas JW, Ma YJ, Lu J, Zhang L, Cao JX, Wu MM, Wang GK, Liu RZ, Liu Z, Zhang SZ, Liu CS, Du LX: Genome-wide analysis reveals adaptation to high altitudes in Tibetan sheep. *Sci Rep,* 6:26670, 2016. DOI: 10.1038/srep26770

2. Liu HJ, Xu TW, Xu SX, Ma L, Han XP, Wang XG, Zhang XL, Hu LY, Zhao N, Chen YW, Pi L, Zhao XQ: Effect of dietary concentrate to forage ratio on growth performance, rumen fermentation and bacterial diversity of Tibetan sheep under barn feeding on the Qinghai-Tibetan plateau. *Peer J,* 7:e7462, 2019. DOI: 10.7717/peerj.7462

3. Nudda A, Atzori AS, Correddu F, Battacone G, Lunesu MF, Cannas A, Pulina G: Effects of nutrition on main components of sheep milk. *Small Ruminant Res,* 184, 106-115, 2020. DOI: 10.1016/j.smallrumres.2019.11.001

4. Addah W, Ayantunde A, Okine EK: Effects of restricted feeding and re-alimentation of dietary protein or energy on compensatory growth of sheep. *S Afr J Anim Sci,* 47 (3): 15-27, 2017. DOI: 10.4314/sajas.v47i3.15

5. Wendorff WL, Haenlein FW: Sheep Milk - Composition and Nutrition. **In,** Park YW, Haenlein GFW, Wendorff WL (Eds): Handbook of Milk of Non-Bovine Mammals. Second ed., 210-221, Wiley, 2017.

6. Giroux JH, Veillette N, Britten M: Use of denatured whey protein in the production of artisanal cheeses from cow, goat and sheep milk. *Small Ruminant Res*, 161, 34-42, 2018. DOI: 10.1016/j.smallrumres.2018.02.006

7. Chleilat F, Klancic T, Ma K, Schick A, Nettleton JE, Reimer RA: Human milk oligosaccharide supplementation affects intestinal barrier function and microbial composition in the gastrointestinal tract of young sprague dawley rats. *Nutrients*, 12 (5): 1532-1544, 2020. DOI: 10.3390/nu12051532

8. Zhang XY, Liu XX, Li FD, Yue XP: The differential composition of whey proteomes in hu sheep colostrum and milk during different lactation periods. *Animals*, 10 (10): 1784-1796, 2020. DOI: 10.3390/ani10101784

9. Konečná L, Kuchtík J, Sedláková M, Šustová K, Filipčík R: The effect of the lactation stage on milk yield, composition and renneting parameters of milk in sheep reared under intensive nutrition. *Acta Univ Agric Silvic Mendelianae Brun*, 67 (8): 85-89, 2019. DOI: 10.11118/actaun201967010085

10. Ferro MM, Tedeschi LO, Atzori AS: The comparison of the lactation and milk yield and composition of selected breeds of sheep and goats. *Transl Anim Sci,* 4 (1): 56-67, 2017. DOI: 10.2527/tas2017.0056

11. Jia JL, Liang CN, Wu XY, Xiong L, Bao PJ, Chen Q, Yan P: Effect of high proportion concentrate dietary on Yak jejunal structure, physiological function and protein composition during cold season. *Sci Rep,* 11:5502, 2021. DOI: 10.1038/s41598-021-84991-3

12. Guo W, Wang WW, Bi SS, Long RJ, Ullah F, Shafiq M, Zhou M, Zhang Y: Characterization of anaerobic rumen fungal community composition in Yak, Tibetan sheep and small tail Han sheep grazing on the Qinghai-Tibetan Plateau. *Animals*, 10 (1): 144-157, 2020. DOI: 10.3390/ani10010144

13. Cui K, Wang B, Ma T, Si BW, Zhang NF, Tu Y, Diao QY: Effects of dietary protein restriction followed by realimentation on growth performance and liver transcriptome alterations of lamb. *Sci Rep,* 8:15185, 2018. DOI: 10.1038/s41598-018-33407-w

14. Zhou JW, Guo YM, Kang JP, Degen AA, Titgemeyer EC, Jing XP, Wang WJ, Shang ZH, Li ZP, Yang G, Long RJ: Tibetan sheep require less energy intake than small-tailed Han sheep for N balance when offered a low protein diet. *Anim Feed Sci Technol,* 248, 85-94, 2019. DOI: 10.1016/j.anifeedsci.2019.01.006

15. Bessho Y: Migration for ecological preservation? Tibetan herders' decision making process in the eco-migration policy of Golok Tibetan autonomous prefecture (Qinghai province, PRC). *Nomadic Peoples*, 19 (2): 189-208, 2015.

16. Yang S: Feed Analysis and Quality Test Technology. 97-108, Beijing Agriculture Press: Beijing, 1993.

17. Jia JL, Jin JP, Chen Q, Yuan Z, Li HQ, Bian JH, Gui LS: Eukaryotic expression, Co-IP and MS identify BMPR-1B protein-protein interaction network. *Biol Res,* 53, 24-37, 2020. DOI: 10.1186/s40659-020-00290-7

18. Ribeiro DM, Planchon S, Leclercq CC, Dentinho MTP, Bessa RJB, Santos-Silva J, Paulos K, Jerónimo E, Renaut J, Almeida AM: The effects of improving low dietary protein utilization on the proteome of lamb tissues. *J Proteomics,* 223:103798, 2020. DOI: 10.1016/j.jprot.2020.103798

19. Soggiu A, Roncada P, Piras C: Proteomics in milk and dairy products. Proteomics in domestic animals: From Farm to Systems Biology. 169-193, Springer, 2018.

20. Piras C, Ceniti C, Hartmane E, Costanzo N, Morittu VM, Roncada P, Britti D, Cramer R: Rapid liquid AP-MALDI MS profiling of lipids and proteins from goat and sheep milk for speciation and colostrum analysis. *Proteomes*, 8 (3): 20-33, 2020. DOI: 10.3390/proteomes8030020

21. Lu J, Zhang SW, Liu L, Pang XY, Ma CL, Jiang SL, Lv JP: Comparative proteomics analysis of human and ruminant milk serum reveals variation in

protection and nutrition. *Food Chem*, 261, 274-282, 2018. DOI: 10.1016/j.foodchem.2018.04.065

22. Opgenorth J, Sordillo LM, Lock AL, Gandy JC, VandeHaar MJ: Colostrum supplementation with n-3 fatty acids alters plasma polyunsaturated fatty acids and inflammatory mediators in newborn calves. *J Dairy Sci,* 103 (22): 11676-11688, 2020. DOI: 10.3168/jds.2019-18045

23. Mohapatra A, Shinde AK, Singh R: Sheep milk: A pertinent functional food. *Small Ruminant Res,* 181, 6-11, 2019. DOI: 10.1016/j.smallrumres.2019.10.002

24. Dror DK, Allen LH: Overview of nutrients in human milk. *Adv Nutr,* 9 (1): 278-294, 2018. DOI: 10.1093/advances/nmy022

25. Adarve Gde L, Morales ER, Manrique JM, Extremera FG, Sanz Sampelayo MR: Milk production and composition in Malagueña dairy goats. Effect of genotype for synthesis of alpha s1-casein on milk production and its interaction with dietary protein content. *J Dairy Res,* 76 (2): 137-143 2009. DOI: 10.1017/S0022029908003798

26. Li Y, Chen D, Li J, Zhang XX, Wang CF, Wang JM: Changes in superoxide dismutase activity postpartum from Laoshan goat milk and factors influencing its stability during processing. *Italian J Anim Sci,* 17 (4): 835-844, 2018. DOI: 10.1080/1828051X.2018.1448306

27. Larsen LB, Wedholm A, Møller HS, Andrén A, Lindmark-Månsson H: Proteomic study of regressions between milk yield and whey protein composition. *J Anim Feed Sci*, 16 (2): 200-206, 2007. DOI: 10.22358/jafs/74194/2007

28. Mizukami J, Takaesu G, Akatsuka H, Sakurai H, Ninomiya-Tsuji J, Matsumoto K, Sakurai N: Receptor activator of NF-κB ligand (RANKL) activates TAK1 mitogen-activated protein kinase kinase kinase through a signaling complex containing RANK, TAB2, and TRAF6. *Mole Cell Biol,* 22 (4): 992-1000, DOI: 10.1128/MCB.22.4.992-1000.2002

29. Navarro F, Galan-Malo P, Pérez MD, Abecia J, Mata L, Calvo M, Sánchez L: Lactoferrin and IgG levels in ovine milk throughout lactation: Correlation with milk quality parameters. *Small Ruminant Res,* 168, 12-18, 2018. DOI: 10.1016/j.smallrumres.2018.09.002

30. Dinc H, Ozkan E, Koban E, Togan I: Beta-casein A1/A2, kappa-casein and beta-lactoglobulin polymorphisms in Turkish cattle breeds. *Arch Anim Breed,* 56, 650-657, 2013. DOI: 10.7482/0003-9438-56-065

31. Miclo L, Roux E, Genay M, Brusseaux E, Poirson C, Jameh N, Perrin C, Dary A: Variability of hydrolysis of β-, α_{s1}-, and α_{s2}-caseins by 10 strains of *Streptococcus thermophilus* and resulting bioactive peptides. *J Agric Food Chem,* 60 (2): 554-565, 2012. DOI: 10.1021/jf202176d

32. Medina Gallardo AL: Behavior of iron fixed on bovine and phosphorylated casein after hydrolysis produced by digestive proteases. *Arch Latinoam Nutr,* 44 (2): 112-116, 1994.

33. Zhang G, Keiderling TA: Equilibrium and dynamic spectroscopic studies of the interaction of monomeric β-lactoglobulin with lipid vesicles at low pH. *Biochemistry,* 53 (19): 3079-3087, 2014. DOI: 10.1021/bi500027x

34. Ingham B, Smialowska A, Kirby NM, Wang C, Carr AJ: A structural comparison of casein micelles in cow, goat and sheep milk using X-ray scattering. *Soft Matter*, 14, 3336-3343, 2018. DOI: 10.1039/C8SM00458G

35. Jawasreh K, Amareen AA, Aad P: Effect and interaction of β-lactoglobulin, kappa casein, and prolactin genes on milk production and composition of Awassi sheep. *Animals*, 9 (6):382, 2019. DOI: 10.3390/ani9060382

36. Zeigler ME, Wicha MS: Posttranscriptional regulation of α-casein mRNA accumulation by laminin. *Exp Cell Res,* 200 (2): 481-489, 1992. DOI: 10.1016/0014-4827(92)90199-I