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Effects of Highland Barley Straw on Body Weight Gain and Immune Indices of Growing Tibetan Sheep (Postprint)

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Abstract

This study used highland barley straw to replace oat hay as the sole roughage source to investigate its effects on body weight gain and immune function in growing Tibetan sheep. Eighteen healthy 4-5 month old valley-type Tibetan sheep were selected and randomly divided into 2 groups: the highland barley group (highland barley straw 300 g/d + concentrate 300 g/d) and the oat group (oat hay 300 g/d + concentrate 300 g/d). The experimental period lasted 4 weeks, with the first week as a preliminary period and weeks 2-4 as the formal experimental period. At the end of the formal experimental period, following the principle of equal numbers of males and females (1/2 each), 6 sheep were selected from each group for slaughter and sampling. The results showed that there were no significant differences ($P>0.05$) in body weight, daily weight gain, spleen weight, and spleen index between the highland barley group and the oat group; there were no significant differences ($P>0.05$) in serum immunoglobulin (Ig) G, IgA, IgM, lysozyme content, and acid phosphatase activity between the two groups, but the lysozyme content in the highland barley group showed a tendency to be higher than that in the oat group ($P=0.083$); there were no significant differences ($P>0.05$) in the contents of interleukin (IL)-2, IL-4, IL-6, IL-10, and interferon-γ (IFN-γ) in serum, thymus, and spleen between the two groups. In summary, compared with oat hay, feeding highland barley straw did not reduce the body weight gain and immune performance of growing Tibetan sheep, and showed potential to increase the content of non-specific immune factors (lysozyme) in the body, thus it can be used as a roughage source for Tibetan sheep.

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Full Text

Effects of Highland Barley Straw on Body Weight Gain and Immune Indexes in Growing Tibetan Sheep

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Abstract: This study investigated the effects of substituting oat hay with highland barley straw as the sole roughage source on body weight gain and immune function in growing Tibetan sheep. Eighteen healthy 4- to 5-month-old Valley Tibetan sheep were selected and randomly divided into two groups: a highland barley group (highland barley straw 300 g/d + concentrate 300 g/d) and an oat group (oat hay 300 g/d + concentrate 300 g/d). The trial lasted for 4 weeks, with the first week as a preliminary period and weeks 2–4 as the formal experimental period. After the formal period, six sheep per group (half males and half females) were selected for slaughter sampling. The results showed no significant differences between groups in body weight, daily gain, spleen weight, or spleen index ($P>0.05$). Serum immunoglobulin (Ig) G, IgA, IgM, lysozyme content, and acid phosphatase activity also showed no significant differences ($P>0.05$), though lysozyme content in the highland barley group exhibited a tendency to be higher than in the oat group ($P=0.083$). Interleukin (IL)-2, IL-4, IL-6, IL-10, and interferon-γ (IFN-γ) contents in serum, thymus, and spleen did not differ significantly between groups ($P>0.05$). Overall, compared with oat hay, feeding highland barley straw did not reduce body weight gain or impair immune performance in growing Tibetan sheep and showed potential for increasing non-specific immune factors (lysozyme). Highland barley straw can serve as a roughage source for Tibetan sheep.

Keywords: highland barley straw; weight gain; immune index; Tibetan sheep
CLC number: S826

Introduction

Roughage is a major constraint to livestock development on the Qinghai-Tibet Plateau. In recent years, the exploitation of highland barley grain resources

has generated substantial quantities of highland barley straw. Developing the feeding value of this locally abundant resource could alleviate the acute contradiction between forage availability and livestock demand. However, in plateau agricultural areas, most highland barley straw is currently discarded or used as fuel, with only a small portion utilized as forage resources for pastoral areas during winter and spring [1-2]. Highland barley straw fibers are finer and shorter than those of common wheat straw, making it more palatable [3], and its nutritional value is moderate yet comprehensive among straw roughages [4-6], giving it broad application prospects as a bulk roughage in the Qinghai-Tibet Plateau region.

Highland barley grain contains 3.66%–8.62% β-glucan, and highland barley straw also has relatively high β-glucan content, reaching up to 0.096% in straw from Tibet [7]. The active structure of β-glucan is a polysaccharide composed of glucose units, mostly linked by β-1,3 and β-1,4 glycosidic bonds. Numerous studies suggest that β-glucan functions are related to the immune function and activity of intestinal epithelial cells and gut-associated lymphoid tissue, promoting small intestinal motility and increasing probiotic populations in the colon [8-11]. β-glucan can activate macrophages, neutrophils, B lymphocytes, and other immune cells, thereby increasing interleukin, cytokine, and specific antibody contents to comprehensively stimulate the immune system and affect immune function [12].

Oat hay is a widely used high-quality forage for ruminants on the Qinghai-Tibet Plateau, with soft texture and high nutritional value [13-14]. Similar to highland barley straw, oat hay is also rich in β-glucan [15]. Long et al. [16] reported that supplementary feeding of highland barley straw improved production performance in grazing yaks. However, relevant research remains limited, and no studies have reported the effects of highland barley straw on immune function in Tibetan sheep. Therefore, this experiment aimed to compare the effects of highland barley straw and oat hay on body weight, immune organ development, and immune performance in growing Tibetan sheep, providing data support for the deep development and utilization of highland barley straw.

1.1 Experimental Design

Eighteen healthy 4- to 5-month-old Valley Tibetan sheep [(16.13$±$2.33) kg] were selected and randomly divided into two groups: a highland barley group (highland barley straw 300 g/d + concentrate 300 g/d) and an oat group (oat hay 300 g/d + concentrate 300 g/d). The trial lasted 4 weeks, with week 1 as a preliminary period and weeks 2–4 as the formal experimental period.

1.2 Feeding Management

Sheep were housed individually and fed twice daily (09:00 and 16:00) with equal portions each time. Chopped roughage (3–5 cm) was provided after concentrate consumption. Residual feed was collected before morning feeding the next day,

and water was available ad libitum. Powdered compound feed was formulated according to Chinese "Feed Standard for Meat Sheep" (NY/T 816-2004) nutritional requirements. Diet composition and nutrient levels are shown in Table 1 .

Table 1 Composition and nutrient levels of diets (DM basis) %

Item	Highland barley group	Oat group
Ingredients		
Highland barley straw	50.0	–
Oat hay	–	50.0
Corn	22.5	22.5
Soybean meal	13.0	13.0
Wheat bran	10.0	10.0
Palm oil	1.0	1.0
$CaCO_3$	1.5	1.5
Premix[1])	1.0	1.0
NaCl	1.0	1.0
Total	100.0	100.0
Nutrient levels[2])		
Dry matter	91.2	91.5
Crude protein	10.8	12.1
Ether extract	2.8	3.1
Acid detergent fiber	33.5	30.2
Neutral detergent fiber	55.3	52.8
Crude ash	6.8	7.2
Calcium	0.8	0.8
Available phosphorus	0.3	0.3

[1]) Per kilogram of premix contained: VA 90,000 IU, VD 20,000 IU, $MgSO_4 \cdot H_2O$ 340 g, $FeSO_4 \cdot 7H_2O$ 5 g, $CuSO_4 \cdot 5H_2O$ 2 g, $MnSO_4 \cdot H_2O$ 6 g, $ZnSO_4 \cdot H_2O$ 10 g, Na_2SeO_3 0.03 g, KI 0.08 g, $CoCl \cdot 6H_2O$ 0.06 g.
[2]) Nutrient levels were measured values.

1.3 Body Weight and Daily Gain Measurement

Body weight was measured before morning feeding on day 1 and the final day of the formal period to calculate daily gain.

1.4 Sample Collection and Analysis

Feed samples were collected during the formal period, mixed, and used to determine dry matter, ether extract, crude protein, acid detergent fiber, crude ash, calcium, and phosphorus content according to Zhang Liying [17].

On the final day of the formal period, blood was collected via jugular venipuncture before morning feeding. Serum was separated by centrifugation at 3,500 r/min (relative centrifugal force 1,100×g) for 10 min and stored at -20°C for analysis of immunoglobulin (Ig) and lysozyme contents, acid phosphatase (ACP) activity, and immune-related cytokine contents. On day 2 after the formal period, 12 sheep (6 per group, half males and half females) were slaughtered. After exsanguination, thymus and spleen tissues were separated, weighed, and sampled. Tissue samples were snap-frozen in liquid nitrogen and stored at -80°C for cytokine analysis.

Spleen index = spleen weight (g) / body weight (kg).

1.4.1 Serum Ig Content Determination Serum IgG, IgA, and IgM contents were determined by enzyme-linked immunosorbent assay (ELISA) using an MB-530 microplate reader (Shenzhen Huisong Technology Development Co., Ltd.) according to kit instructions (Wuhan Huamei Bioengineering Co., Ltd.).

1.4.2 Serum Lysozyme Content and Acid Phosphatase Activity Determination Lysozyme content was determined by ELISA (MB-530 microplate reader) according to kit instructions (Wuhan Huamei Bioengineering Co., Ltd.). Acid phosphatase activity was determined by ELISA (Infinite M200 PRO microplate reader, Tecan, Switzerland) according to kit instructions (Nanjing Jiancheng Bioengineering Institute).

1.4.3 Cytokine Content Determination in Serum and Immune Organs IL-2, IL-4, IL-6, IL-10, and interferon-γ (IFN-γ) contents in serum, thymus, and spleen were determined by ELISA (MB-530 microplate reader) according to kit instructions (Wuhan Huamei Bioengineering Co., Ltd.).

1.5 Statistical Analysis

After removing extreme values using Dixon and Grubbs tests, data were analyzed using SPSS 19.0. A general linear model (GLM) was employed with treatment as a fixed factor and animal body weight as a covariate. Treatment effects were analyzed at Alpha=0.05.

2.1 Body Weight, Daily Gain, Spleen Weight, and Spleen Index

As shown in Table 2 , no significant differences were observed between the highland barley and oat groups in body weight, daily gain, spleen weight, or spleen index ($P>0.05$).

Table 2 Effects of highland barley straw and oat hay on body weight, daily gain, spleen weight, and spleen index in Tibetan sheep

Item	Highland barley group	Oat group	P-value
Initial body weight (kg)	16.17$±2.84\|16.08±1.96\|—\|\|$Finalbodyweight(kg)$\|20.69±2.57\|20.73±0.62\|—\|\|$Dailygain(g/d)$|215.4	>0.05	
	0.05||$Spleenweight(g)$|38.68±9.56|42.24±8.11| >		
	0.05||$Spleenindex(g/kg)$|1.85±0.30|2.04±$0.43		

2.2 Serum Ig, Lysozyme Content, and Acid Phosphatase Activity

As shown in Table 3 , no significant differences were found between groups in serum IgG, IgA, IgM, lysozyme content, or acid phosphatase activity (P>0.05). However, lysozyme content in the highland barley group showed a tendency to be higher than in the oat group (P=0.083).

Table 3 Effects of highland barley straw and oat hay on contents of Igs and lysozyme and ACP activity in serum of Tibetan sheep

Item	Highland barley group	Oat group	P-value
IgG (mg/mL)	69.48$±23.97|73.95±13.94| >	0.083	
	0.05||$IgA(mg/mL)$|5.44±2.28|7.19±3.56| >		
	0.05||$IgM(mg/mL)$|1.07±0.48|0.99±0.46| >		
	0.05||$ACP(U/mL)$|2.20±0.79|1.11±0.89| >		
	0.05||$Lysozyme(\mu g/mL)$|5.88±0.76|8.13±$2.23		

2.3 Cytokine Contents in Serum, Thymus, and Spleen

As shown in Table 4 , no significant differences were observed between groups in IL-2, IL-4, IL-6, IL-10, and IFN-γ contents in serum, thymus, or spleen (P>0.05). Numerically, all cytokine contents (IL-2, IL-4, IL-6, IL-10, and IFN-γ) in thymus were higher in the highland barley group than in the oat group, while in spleen, all these cytokine contents were lower in the highland barley group.

Table 4 Effects of highland barley straw and oat hay on contents of IL-2, IL-4, IL-6, IL-10, and IFN-γ in serum, thymus, and spleen of Tibetan sheep

Item	Tissue	Highland barley group	Oat group	P-value
IL-2	Serum (pg/mL)	26.96$±12.78\|31.89±16.86\| >Serum (pg/mL)\|51.62±7.46\|43.04±7.87\| >0.05\|\|\|$Thymus(ng/g)$\|24.87±7.24\|29.16±5.08\| >0.05\|\|\|$Spleen(ng/g)$\|14.20±12.46\|6.65±2.39\| >0.05\|\|\|$Thymus(ng/g)$\|42.19±16.02\|30.91±4.67\| >0.05\|\|\|$Spleen(ng/g)$\|12.26±4.24\|14.57±5.22\| >0.05\|\|$IL-4$\|$Serum(pg/mL)$\|1.00±0.72\|1.09±1.09\| >0.05\|\|\|$Thymus(ng/g)$\|0.83±0.16\|0.76±0.14\| >0.05\|\|\|$Spleen(ng/g)$\|0.42±0.07\|0.44±0.09\| >0.05\|\|$IL-10$\|$Serum(pg/mL)$\|32.64±1.80\|33.35±2.52\| >0.05\|\|\|$Thymus(ng/g)$\|68.66±13.91\|65.00±27.06\| >0.05\|\|\|$Spleen(ng/g)$\|17.80±3.64\|19.15±2.46\| >0.05\|\|$IFN-$	5.08$±3.47\|3.87±1.55\| >0.05\|\|\|$Thymus(ng/g)$|7.88±2.24|6.15±0.86

3.1 Effects on Body Weight, Daily Gain, Spleen Weight, and Spleen Index

Both highland barley straw and oat hay serve as major forage resources in the Qinghai-Tibet Plateau region. Long et al. [16] found that supplementary feeding of highland barley straw reduced weight loss in grazing yaks during the cold season. Long et al. [18] also reported that supplementary highland barley straw increased calving rates by 19% and improved hormone levels in yak cows. However, no studies have investigated the feeding value of highland barley straw in sheep. In our trial, the measured crude protein content of highland barley straw was 1.42%, lower than that of oat hay (4.34%). Neutral detergent fiber and acid detergent fiber contents in highland barley straw were 70.9% and 45.8%, respectively, higher than those in oat hay (59.7% and 38.7%). However, ether extract content was similar between the two (1.2% vs. 1.7%). These values are consistent with other studies [4-6], indicating that oat hay has superior overall nutritional value. Nevertheless, under the condition of supplementing 300 g/d concentrate per lamb, using either highland barley straw or oat hay as the sole roughage source resulted in no significant differences in body weight or daily gain, demonstrating that highland barley straw can serve as a roughage source for growing Tibetan sheep.

Immune organ weight can be used as an evaluation index of immune function, while organ index often reflects physiological status. This trial found no significant differences in spleen weight or spleen index between Tibetan sheep fed highland barley straw or oat hay. As a functional polysaccharide, most β-glucan cannot be digested and absorbed. In monogastric animals, it is mainly fermented by microbial flora in the large intestine and excreted in feces, though

small amounts can be absorbed into tissues such as liver, heart, and kidney [9]. Small quantities of β-glucan may not affect overall immune function [19]. Most β-glucan immune function research has focused on pigs, poultry, rodents, and aquatic animals [10-11,20], with limited studies in ruminants. The digestion, absorption, and metabolism mechanisms of β-glucan in ruminants remain poorly understood. Although both highland barley straw and oat hay contain high levels of β-glucan, the amount absorbed from roughage in this form is likely limited, which may explain why no significant differences in spleen development were observed between the two dietary treatments.

3.2 Effects on Serum Ig, Lysozyme Content, and Acid Phosphatase Activity

IgG and IgM are primary effector molecules of humoral immunity, while IgA is the main effector of mucosal immune responses. Levels of these three antibodies are important indicators of immune status. Acid phosphatase and lysozyme primarily reflect non-specific immune status. Zhou et al. [21-22] reported that adding 75 mg/kg yeast β-glucan to diets of weaned calves significantly increased serum IgG and IgM contents but did not affect IgA content, with lysozyme content increasing as dietary β-glucan levels rose. Dietary yeast β-glucan supplementation in lambs increased serum IgG content [23] and regulated non-specific immunity in mammary glands [24]. *Allium mongolicum* polysaccharide also increased secretory IgA content in goats, improved intestinal mucosal morphology and microflora, and enhanced immune function [25]. In contrast, our trial found no significant changes in serum IgG, IgA, IgM, lysozyme content, or acid phosphatase activity in Tibetan sheep fed highland barley straw compared with oat hay. This discrepancy may be due to lower β-glucan content in roughage compared with previous studies, as well as differences in polysaccharide types and structures, leading to different immunological effects.

However, lysozyme content in the highland barley group showed a tendency to increase compared with the oat group. Lysozyme can kill bacteria by hydrolyzing β-1,4 glycosidic bonds in bacterial cell wall peptidoglycan, causing cell wall dissolution, thereby directly enhancing antibacterial and antiviral capacity. Additionally, lysozyme can be converted into digestive enzymes in the ruminant forestomach [26], facilitating bacterial lysis to obtain microbial protein and improving nutrient supply, which indirectly enhances adaptability.

3.3 Effects on Cytokine Contents in Serum, Thymus, and Spleen

β-glucan can activate lymphocytes and participate in immune responses by releasing pro-inflammatory cytokines, stimulating B lymphocytes to produce TNF-α, IL-6, and IL-8 [19]. Dietary yeast β-glucan supplementation in lambs increased serum IFN-γ content [23]. However, this trial found that substituting oat hay with highland barley straw did not affect IL-2, IL-4, IL-6, IL-10, or IFN-γ contents in thymus and spleen. Numerically, all cytokine contents in thymus were higher in the highland barley group, while all were lower in spleen com-

pared with the oat group. This may indicate different regulatory mechanisms of the cytokine network in different immune organs between the two roughage sources, possibly related to differences in β-glucan structure [27-28], warranting further investigation.

Conclusion

Feeding highland barley straw instead of oat hay does not affect body weight gain or immune performance in growing Tibetan sheep and shows potential for increasing non-specific immune factors (lysozyme). When combined with appropriate concentrate feed, highland barley straw can serve as a roughage source in Tibetan sheep production systems.

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