

## Effects of Dietary Nutrient Restriction on Growth Performance and Visceral Organ Development in Early-Weaned Hu Sheep Lambs: Postprint

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### Abstract

This study aimed to investigate the effects of dietary nutrient restriction on growth performance and visceral organ development in early-weaned Hu sheep lambs. Sixty-four 17-day-old Hu sheep lambs were randomly allocated to 4 groups: control (CON), 20% protein restriction (PR), 20% energy restriction (ER), and 20% both protein and energy restriction (BR), with 4 replicates per group and 4 lambs per replicate, half male and half female. The preliminary period lasted 4 days, followed by a 40-day formal experimental period. Feed intake of starter feed and milk replacer was recorded, and one lamb per replicate was slaughtered at 40 and 60 days of age to measure visceral organ weights. The results showed: 1) The average daily gain of PR and BR groups at 21-40 days of age, and PR, ER, and BR groups at 41-60 days of age was significantly lower than that of the CON group ( $P < 0.05$ ); the feed conversion ratio of the experimental groups at 21-40 days of age, and PR and BR groups at 41-60 days of age was significantly higher than that of the CON group ( $P < 0.05$ ). 2) At 40 days of age, liver weight and its proportion to pre-slaughter live weight, and rumen weight and its proportion to complex stomach weight in PR, ER, and BR groups were significantly lower than those in the CON group ( $P < 0.05$ ); at 60 days of age, liver weight and its proportion to pre-slaughter live weight, and rumen weight and its proportion to complex stomach weight in ER and BR groups were significantly lower than those in PR and CON groups ( $P < 0.05$ ). In conclusion, protein restriction inhibited growth performance of weaned lambs at 21-60 days of age and suppressed visceral organ development during the early weaning period (21-40 days of age), while energy restriction inhibited visceral organ development in weaned lambs, particularly the development of liver and rumen.

## Full Text

### Abstract

This study investigated the effects of dietary nutritional restriction on growth performance and visceral organ development in early-weaned Hu lambs. Sixty-four 17-day-old Hu lambs were randomly divided into four groups: a control (CON) group, a 20% protein restriction (PR) group, a 20% energy restriction (ER) group, and a 20% combined energy and protein restriction (BR) group. Each group comprised four replicates with four lambs per replicate (half male and half female). The pretest lasted 4 days, followed by a 40-day experimental period. Intakes of milk replacer and starter feed were recorded, and all lambs were weighed at 20, 40, and 60 days of age. Subsequently, one lamb per replicate was slaughtered at 40 and 60 days of age for visceral organ weighing. The results showed: (1) Average daily gain (ADG) of lambs at 21-40 days of age in the PR and BR groups was significantly lower than in the CON group ( $P < 0.05$ ). ADG at 41-60 days of age and overall ADG at 21-60 days of age in the PR, ER, and BR groups were significantly lower than in the CON group ( $P < 0.05$ ). The feed-to-gain ratio of lambs at 21-40 days of age in all experimental groups and at 41-60 days of age in the PR and BR groups was significantly higher than in the CON group ( $P < 0.05$ ). (2) At 40 days of age, liver weight and its percentage of live weight before slaughter (LWBS), as well as rumen weight and its percentage of complex stomach weight, were significantly decreased in the PR, ER, and BR groups compared to the CON group ( $P < 0.05$ ). At 60 days of age, these parameters were significantly decreased in the ER and BR groups compared to the PR and CON groups ( $P < 0.05$ ). In conclusion, protein restriction reduced growth performance (21-60 days of age) and visceral organ development (21-40 days of age), while energy restriction impaired visceral organ development, particularly in the liver and rumen, of early-weaned lambs.

**Keywords:** lamb; nutritional restriction; growth performance; organ development

## 1. Materials and Methods

### 1.1 Experimental Period and Location

The experiment was conducted from November to December 2014 at Taizhou Hailun Sheep Farming Co., Ltd., Jiangsu Province, China.

### 1.2 Experimental Animals and Design

Sixty-four 17-day-old Hu lambs with similar body weight [ $(2.5 \pm 0.2)$  kg] were randomly divided into four groups: control (CON), 20% protein restriction (PR), 20% energy restriction (ER), and 20% combined energy and protein restriction (BR). Each group consisted of four replicates with four lambs per replicate (half male and half female). The pretest lasted 4 days, and the formal experiment lasted 40 days. Lambs in the CON group were fed ad libitum, while those in the

experimental groups were subjected to nutritional restriction. The PR group received 20% protein restriction, the ER group received 20% energy restriction, and the BR group received 20% combined energy and protein restriction. The experimental period comprised 4 days of adaptation followed by 40 days of data collection, with lambs weighed at 20, 40, and 60 days of age. The total experimental duration was 44 days. Lambs in the CON group had free access to milk replacer and starter feed, while those in the PR, ER, and BR groups received restricted amounts of milk replacer and starter feed according to their respective restriction protocols.

### 1.3 Diet Composition

The nutrient levels of the experimental diets are shown in Tables 1 and 2. The milk replacer nutrient levels were formulated according to WANG [13] and the Feeding Standard of Sheep (NY/T 816-2004) [14]. Nutrient levels were measured values except for metabolizable energy (ME), which was calculated based on these references. The starter feed composition and nutrient levels are presented in Table 2.

### 1.4 Feeding Management

Lambs were weaned at 17 days of age and transferred to individual pens. After a 3-day adaptation period, they were fed ad libitum with milk replacer and starter feed. During days 1-20 post-weaning, starter feed was provided at 1/3 of the milk replacer amount. From day 21 onward, starter feed was gradually increased while milk replacer was decreased. During days 21-50 and 51-60, starter feed amounts were set at 2.0% and 1.5% of lamb body weight, respectively. From days 21-30, lambs were fed three times daily (08:00, 12:00, 18:00); from days 31-60, they were fed twice daily (09:00, 18:00). The milk replacer-to-water ratio was 1:5 (w/v) at 50°C, with a feeding temperature of 40°C [15].

All lambs were housed in individual pens from 17 days of age with free access to water. Pens were cleaned daily, and strict hygiene protocols were maintained. Vaccinations and deworming were administered according to standard farm practices, with each lamb receiving 0.5 mL of vitamin AD injection and 0.1 mL of selenium supplement once.

### 1.5 Measurement Indicators

**Diet nutrient levels:** Determined using standard methods. Dry matter (DM), crude protein (CP), ether extract (EE), and ash were measured using proximate analysis. Calcium (Ca) and total phosphorus (TP) were analyzed according to standard procedures [16].

**Growth performance:** Recorded as average daily gain (ADG) and feed intake. Milk replacer intake and starter intake were measured daily.

**Visceral organ indices:** At 40 and 60 days of age, four lambs per group (one

per replicate) were slaughtered after a 16-hour fast [17] to measure live weight before slaughter (LWBS). Organ weights including heart, liver, spleen, lung, and kidney were recorded. The rumen, reticulum, omasum, and abomasum were separated, weighed, and their relative weights calculated. Small and large intestines were measured for length and weight.

Organ indices were calculated as: - Organ weight percentage (% of LWBS) = (organ weight / LWBS)  $\times$  100 - Organ weight percentage (% of total complex stomach weight) = (organ weight / total complex stomach weight)  $\times$  100

## 1.6 Statistical Analysis

Data were processed using Excel and analyzed using SAS 8.1 software. One-way ANOVA was performed, and Duncan's multiple range test was used for post-hoc comparisons. Differences were considered significant at  $P < 0.05$  and marginally significant at  $0.05 > P > 0.10$ .

## 2. Results

### 2.1 Growth Performance

As shown in Table 3, ADG of lambs in the PR and BR groups at 21-40 days of age was significantly lower than in the CON group ( $P < 0.05$ ). ADG at 41-60 days of age and overall ADG at 21-60 days of age in the PR, ER, and BR groups were significantly lower than in the CON group ( $P < 0.05$ ). Milk replacer intake did not differ significantly among groups ( $P > 0.05$ ). Starter intake in the PR, ER, and BR groups at 21-60 days of age was significantly lower than in the CON group ( $P < 0.05$ ). The feed-to-gain ratio in all experimental groups at 21-40 days of age and in the PR and BR groups at 41-60 days of age was significantly higher than in the CON group ( $P < 0.05$ ).

### 2.2 Visceral Organ Development

As shown in Table 4, at 40 days of age, heart, liver, spleen, lung, and kidney weights in the ER, PR, and BR groups were significantly lower than in the CON group ( $P < 0.05$ ). Liver weight percentage of LWBS was significantly lower in the ER, PR, and BR groups compared to the CON group ( $P < 0.05$ ), while heart and kidney weight percentages were marginally lower ( $0.05 > P > 0.10$ ). At 60 days of age, heart, liver, spleen, lung, and kidney weights in the ER, PR, and BR groups showed no significant differences from the CON group ( $P > 0.05$ ). However, liver weight percentage of LWBS was significantly lower in the ER and BR groups compared to the PR and CON groups ( $P < 0.05$ ), and kidney weight percentage was significantly lower in the ER group compared to the CON and PR groups ( $P < 0.05$ ).

### 2.3 Complex Stomach Development

As shown in Table 5, at 40 days of age, rumen weight in the PR, ER, and BR groups was significantly lower than in the CON group ( $P < 0.05$ ). Reticulum and omasum weights in the BR group were significantly lower than in the CON group ( $P < 0.05$ ). Total complex stomach weight in the PR, ER, and BR groups was significantly lower than in the CON group ( $P < 0.05$ ). The rumen weight percentage of total complex stomach weight was significantly lower in the PR, ER, and BR groups compared to the CON group ( $P < 0.05$ ), while the abomasum percentage was significantly higher in the BR group compared to the other three groups ( $P < 0.05$ ). At 60 days of age, rumen weight in the CON group was significantly higher than in the other three groups ( $P < 0.05$ ), while reticulum, omasum, and abomasum weights did not differ significantly among groups ( $P > 0.05$ ). The rumen weight percentage of total complex stomach weight was significantly lower in the BR group compared to the CON group ( $P < 0.05$ ), while the abomasum percentage was significantly higher in the BR group compared to the PR and CON groups ( $P < 0.05$ ).

### 2.4 Intestinal Tract Development

As shown in Table 6, at 40 days of age, small intestine weight in the PR, ER, and BR groups was significantly lower than in the CON group ( $P < 0.05$ ). Large intestine weight in the ER and BR groups was significantly lower than in the CON group ( $P < 0.05$ ). The small intestine weight percentage of LWBS was significantly lower in the PR, ER, and BR groups compared to the CON group ( $P < 0.05$ ). At 60 days of age, small intestine weight in the ER group was significantly lower than in the other three groups ( $P < 0.05$ ), while large intestine weight did not differ significantly among groups ( $P > 0.05$ ). The small intestine weight percentage of LWBS was significantly lower in the ER group compared to the CON group ( $P < 0.05$ ), with no significant differences among the other groups ( $P > 0.05$ ).

## 3. Discussion

### 3.1 Effects on Growth Performance

Growth performance is a primary indicator reflecting animal nutritional status [19]. Lamb growth performance is influenced by multiple factors including breed, age, and nutrition [1]. Previous studies have shown that nutritional restriction negatively affects lamb growth performance [2]. Research by YANG et al. [3], XU et al. [4], and ZHANG et al. [5] demonstrated that both energy and protein restriction reduce lamb ADG and feed efficiency. This study found that protein restriction during 21-40 days of age significantly reduced ADG, consistent with WANG et al. [13] and Sevi et al. [23]. The reduction in ADG may be attributed to decreased nutrient intake limiting protein synthesis and cell proliferation. Energy restriction also reduced ADG, particularly during 41-60 days of age, possibly due to insufficient energy for maintenance and growth

requirements. Combined energy and protein restriction showed the most severe effects, reducing ADG by 27.5% during 21–40 days and 25.6% during 41–60 days compared to the CON group. These results indicate that early post-weaning nutritional restriction significantly impairs growth performance, with protein restriction having more pronounced effects during the early stage and energy restriction becoming more critical during the later stage.

### 3.2 Effects on Visceral Organ Development

The liver is the primary site for nutrient metabolism and conversion. Liver development is closely related to nutrient intake levels and metabolic demands [24]. Protein restriction reduces liver protein synthesis and hepatocyte proliferation, consequently decreasing liver weight [25]. This study found that protein restriction significantly reduced liver weight and its percentage of LWBS at 40 days of age, consistent with previous research [6, 9]. Energy restriction also impaired liver development, particularly at 60 days of age, likely due to insufficient energy for hepatic metabolic processes. The spleen and kidney showed similar responses to nutritional restriction, with reduced weights at 40 days of age but partial recovery by 60 days, suggesting adaptive mechanisms. Heart and lung development were less affected, indicating their higher priority for nutrient allocation during restriction periods. These findings demonstrate that visceral organ development is sensitive to nutritional status, with the liver being most responsive to both protein and energy restriction.

### 3.3 Effects on Complex Stomach Development

The rumen undergoes rapid development during 21–56 days of age, with its functional development directly influencing nutrient utilization efficiency [28]. Protein restriction significantly reduced rumen weight and its proportion of total complex stomach weight at 40 days of age, consistent with studies by BALDWIN et al. [24] and YANG et al. [6]. Energy restriction also impaired rumen development, likely by limiting the energy available for rumen epithelial cell proliferation and papillae development. The reticulum and omasum showed similar but less severe responses. Interestingly, the abomasum weight percentage increased in the BR group, possibly reflecting compensatory development or relative proportion changes due to greater inhibition of fore-stomach development. By 60 days of age, differences in reticulum, omasum, and abomasum weights among groups diminished, suggesting catch-up growth potential, while rumen development remained compromised in restricted groups. These results indicate that early nutritional restriction, particularly protein restriction, can have lasting effects on rumen development, which may permanently affect feed utilization efficiency.

### 3.4 Effects on Intestinal Tract Development

The small intestine is the primary site for nutrient absorption, and its development is crucial for animal growth [37]. Small intestine development is regulated

by multiple factors including nutrient intake, hormones, and growth factors such as insulin-like growth factor-1 (IGF-1) [29]. This study found that protein restriction significantly reduced small intestine weight at 40 days of age, consistent with previous reports [9, 26]. Energy restriction also impaired small intestine development, with effects persisting until 60 days of age, possibly due to reduced IGF-1 levels and limited energy for intestinal epithelial cell proliferation [30-32]. The large intestine was less affected than the small intestine, consistent with its lower metabolic activity and nutrient absorption function. The small intestine weight percentage of LWBS was significantly reduced in restricted groups, indicating disproportionate inhibition of intestinal development relative to body weight. These findings suggest that nutritional restriction during early life can have long-lasting effects on intestinal development and function, potentially affecting lifetime nutrient absorption capacity and growth performance.

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