Effects of Dietary Concentrate Ratio in Late-Gestation Ewes on Growth Performance, Digestive Performance, and Serum Antioxidant Indices of Lambs (Postprint)

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Abstract

This experiment was conducted to investigate the effects of dietary concentrate ratio in late-gestation ewes on growth performance, digestive performance, and serum antioxidant indices of lambs. Sixty-six primiparous Hu sheep at 90 d of gestation with an average body weight of (44.45$±$2.20) kg were randomly assigned to 3 groups based on similar body weight, with 11 replicates per group and 2 animals per replicate. The dietary concentrate ratios for ewes during gestation in each group were 50%, 40%, and 30%, respectively; after parturition, the ewes were fed the same total mixed ration (TMR). At 10 days of age, one lamb per ewe was weaned from maternal milk and fed milk replacer; starter feed was supplemented at 15 days of age; alfalfa hay was supplemented at 20 days of age and fed ad libitum until 60 days of age. Lamb body weight was measured every 10 d, a digestion and metabolism trial was conducted at 51-60 days of age, and lamb blood was collected at 20 and 60 days of age to determine serum antioxidant indices. The results showed that dietary concentrate ratio in late-gestation ewes had no significant effect on lamb body weight at 1, 10, 20, 30, 40, 50, and 60 days of age, body measurement indices at 20 and 60 days of age, or apparent nutrient digestibility (P>0.05). With decreasing dietary concentrate ratio in ewes, serum total antioxidant capacity (T-AOC) and superoxide dismutase (SOD) activity in 20-day-old lambs increased extremely significantly (P<0.01), and the 50% group was significantly or extremely significantly lower than the other two groups at 60 days of age (P<0.05 or P<0.01); the 30% group had extremely significantly higher serum glutathione peroxidase (GSH-Px) activity in lambs at both 20 and 60 days of age than the other two groups (P<0.01); serum malondialdehyde (MDA) content decreased extremely significantly with decreasing dietary concentrate ratio in ewes at 20 days of age (P<0.01), and the 50% group was significantly higher than the 30% group at

60 days of age (P<0.05). The results suggest that dietary concentrate ratio in late-gestation ewes had no significant effect on body weight, body measurement indices, or apparent nutrient digestibility of early-weaned lambs, but serum antioxidant capacity of lambs improved with decreasing dietary concentrate ratio in ewes.

Full Text

Effects of Dietary Concentrate Proportion of Ewes during Late Gestation on Growth Performance, Digestion Performance and Serum Antioxidant Capacity of Lambs

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Abstract: This experiment aimed to investigate the effects of dietary concentrate proportion of ewes during late gestation on growth performance, digestion performance, and serum antioxidant capacity of lambs. Sixty-six primiparous Hu ewes at 90 days of pregnancy with an average body weight of (44.45$±$2.20) kg were randomly allocated to 3 groups according to similar body weight, with 11 replicates per group and 2 ewes per replicate. The concentrate proportions in gestation diets were 50%, 40%, and 30% for the three groups, respectively. After parturition, all ewes were fed the same total mixed ration (TMR). At 10 days of age, one lamb per ewe was selected, weaned from maternal milk, and fed milk replacer until 60 days of age. Lambs were offered starter feed at 15 days of age and alfalfa hay at 20 days of age ad libitum. Lamb body weight was measured every 10 days. A digestion and metabolism trial was conducted from 51 to 60 days of age, and blood samples were collected at 20 and 60 days of age to determine serum antioxidant indices.

The results showed that dietary concentrate proportion of ewes during late gestation had no significant effects on lamb body weight at 1, 10, 20, 30, 40, 50, and 60 days of age, body measurements, or nutrient apparent digestibility at 20 and 60 days of age (P>0.05). However, with decreasing maternal dietary concentrate proportion, serum total antioxidant capacity (T-AOC) and superoxide dismutase (SOD) activity of lambs at 20 days of age increased significantly (P<0.01), while at 60 days of age, the 50% group was significantly or extremely significantly lower than the other two groups (P<0.05 or P<0.01). Serum glutathione peroxidase (GSH-Px) activity at both 20 and 60 days of age in the 30% group was significantly higher than in the other two groups (P<0.01). Serum malondialdehyde (MDA) content at 20 days of age decreased significantly with decreasing maternal dietary concentrate proportion (P<0.01), and at 60 days of age, the 50% group was significantly higher than the 30% group (P<0.05). These results indicate that while dietary concentrate proportion during late

gestation had no significant effects on body weight, body measurements, or nutrient apparent digestibility of early-weaned lambs, decreasing maternal dietary concentrate proportion improved serum antioxidant capacity in lambs.

Key words: pregnancy ewe; concentrate; early weaning; lamb; Hu Sheep

Forage-based feeding systems may pose risks of insufficient nutrient or energy intake in animals. In small- to medium-scale sheep farms or grazing systems, the most common difference between low-input and high-input feeding is the level of concentrate supplementation. Reducing concentrate and increasing various forages from agricultural or pastoral areas is a common cost-saving approach. However, late gestation is a critical period for fetal development in the maternal body, and maternal nutrition during this period may affect postnatal health and development of lambs. Early weaning of lambs can promote digestive system development, and using milk replacer helps block disease transmission from dams, improve survival rates of lambs from prolific ewes, and accelerate breeding of superior breeding stock. The industrialization and intensification of Hu sheep production objectively require early weaning of lambs to shorten the reproductive interval of ewes. Therefore, studying the effects of dietary concentrate proportion of ewes during late gestation on the growth and development of early-weaned lambs is of great significance.

Numerous studies have investigated the effects of maternal nutrition during gestation on offspring development, revealing the role of maternal nutrition through different methods of regulating dietary nutrient levels. Taylor et al., Campion et al., and He et al. studied the effects of maternal energy level during gestation on postnatal development of calves and lambs, while Gao Feng et al. and Su Guoqi et al. examined the effects of feeding level during late gestation in ewes and sows on offspring development. All these studies demonstrated that maternal nutrition plays an important role in offspring development. In addition to directly altering dietary energy and protein levels or feeding amount, animal nutrition levels can also be regulated by adjusting concentrate feeding amount or proportion. Horn et al. adjusted daily concentrate feeding amount in cows to study its effects on lactation performance, reproductive performance, and metabolic responses, while Muhammad et al. adjusted dietary concentrate proportion to investigate its effects on calf intake and growth. Typically, concentrate has higher nutritional value than forage, so changing concentrate feeding amount affects nutrient intake and consequently animal performance.

Hu sheep is an excellent local breed in China, characterized by high prolificacy, early sexual maturity, fast growth rate, suitability for housed feeding, and good milk performance, making it an important breed for intensive housed sheep production. Therefore, achieving reasonable nutritional regulation for Hu ewes during late gestation is significant for the development of China' s mutton sheep industry. Current research on the effects of late gestation ewe nutrition on offspring growth and development has mainly focused on energy, protein, feeding amount, and feed additives, with few reports on effects mediated by adjusting dietary concentrate proportion. Combining the increased nutritional

requirements of ewes during late gestation, this study explored the effects of adjusting dietary concentrate proportion during late gestation on lamb growth performance, digestion performance, and serum antioxidant indices to provide reference for ewe feeding during late gestation in production practice.

1.1 Experimental Time and Location

This experiment was conducted from July 31, 2015, to February 28, 2016, at Linqing Runlin Animal Husbandry Co., Ltd. in Shandong Province.

1.2 Experimental Design

Sixty-six Hu ewes at 90 days of pregnancy with an average body weight of (44.45$±$2.20) kg were selected as experimental animals. At 90 days of gestation, ewes were randomly divided into 3 groups according to similar body weight, with 11 replicates per group and 2 ewes per replicate. The concentrate proportions in diets were 50% (50% group), 40% (40% group), and 30% (30% group) on a dry matter basis, with corresponding forage proportions of 50%, 60%, and 70%. Concentrate and forage were fed separately until parturition. After parturition, ewes were fed the same total mixed ration (TMR).

At 140 days of gestation, 3 ewes per group were selected for a slaughter trial (outside the scope of this experiment, only used to explain animal numbers). One ewe in the 40% group aborted, and 2 ewes in the 30% group aborted or had dead lambs. Ewes gave birth to 108 live lambs (50% group: 20 male, 18 female; 40% group: 19 male, 17 female; 30% group: 15 male, 19 female). At 10 days of age, 3 lambs per group were selected for a slaughter trial (outside the scope of this experiment, only used to explain animal numbers). The remaining lambs available for subsequent experiments were: 50% group with 7 male and 9 female lambs; 40% group with 8 male and 8 female lambs; 30% group with 6 male and 10 female lambs. Average birth weights were 3.34 kg for the 50% group, 3.23 kg for the 40% group, and 3.14 kg for the 30% group.

Lambs were kept with their dams until 10 days of age, when one lamb per ewe was selected for early weaning (selected according to average birth weight and sex distribution within each group) and fed milk replacer until 60 days of age. Lambs were offered starter feed at 15 days of age and alfalfa hay ad libitum at 20 days of age. From 51 to 60 days of age, 4 lambs per group were randomly selected for a digestion and metabolism trial using the total fecal collection method, with a 5-day preliminary period and a 5-day formal collection period.

1.3 Experimental Diets

The diet for the 50% group was formulated based on the nutrient levels for late gestation ewes from Lou Can et al. in our research group, with separate formulation of concentrate and forage. The concentrate consisted of corn, soybean meal, wheat bran, and premix formulated into pellets. The composition and nutrient levels of the concentrate for late gestation ewes are shown in Table 1

. The forage consisted of whole-plant corn silage and peanut straw mixed at a 1:1 dry matter ratio and fed daily after mixing. The concentrate and forage were identical across all three groups. Diet composition and nutrient levels are shown in Table 2 . Milk replacer for weaned lambs was provided by Beijing Precision Animal Nutrition Research Center. Nutrient levels of milk replacer, starter feed, and alfalfa hay are shown in Table 3 .

1.4 Animal Management

The sheep house was a double-row semi-open barn with good ventilation. All experimental sheep were ear-tagged and followed the farm' s regular immunization and disinfection procedures. From 90 days of gestation to parturition, ewes were housed 2 per pen and fed quantitatively. The average dry matter intake of the lowest intake group (30% group, 1.25 kg per ewe) was used as the single ewe feeding amount for all other groups to ensure identical dry matter intake across treatments. Ewes were fed twice daily at 06:30 and 16:00, with forage offered first followed by concentrate after forage was mostly consumed. After parturition, ewes were fed the same TMR ad libitum. Lambs were kept with their dams from 1 to 10 days of age, then weaned from maternal milk from 11 to 60 days of age (Chai Jianmin et al. showed that 10-day weaning is most beneficial for lamb growth and development). Milk replacer feeding amounts were 2.0% and 1.5% of body weight during 11-50 days and 51-60 days of age, respectively. Milk replacer was fed three times daily (07:00, 14:00, and 21:00) by mixing with boiling water cooled to 50 °C at a milk replacer:water ratio of 6:1, then cooled to approximately 40 °C before feeding. Lambs were trained to eat the same starter feed beginning at 15 days of age and offered alfalfa hay ad libitum starting at 20 days of age. Fresh water was available at all times.

1.5 Sample Collection

Lambs were weighed individually before morning feeding every 10 days after birth. During the digestion and metabolism trial at 51-60 days of age, 4 male lambs per group were selected and placed in metabolic cages for total fecal collection, with a 5-day preliminary period and 5-day formal collection period. During the formal collection period, daily feed intake of milk replacer, starter feed, and alfalfa hay, as well as daily fecal output, were recorded accurately. Fecal samples were collected at 10% of total fecal weight, and 10 mL of 10% dilute hydrochloric acid was added per 100 g fresh feces for nitrogen fixation, then stored at -20 °C until analysis.

Blood samples were collected from the jugular vein before morning feeding at 20 and 60 days of age from 8 lambs per group (half male and half female). Blood was centrifuged at 3,000 r/min for 10 min, and serum was collected in 1.5 mL centrifuge tubes and stored at -20 °C until analysis.

1.6 Measurements

Growth performance: Lambs were weighed at 1, 10, 20, 30, 40, 50, and 60 days of age. Due to lamb mortality during the experiment, final lamb numbers were 13 (50% group), 11 (40% group), and 13 (30% group). Body measurements including body height, chest depth, chest circumference, abdominal circumference, head width, head length, straight crown-rump length, curved crown-rump length, and body length were determined at 20 and 60 days of age according to the methods described by Zhang Chongzhi.

Sample composition analysis: Diets (ewe concentrate, forage, lamb milk replacer, starter feed, alfalfa), and fecal samples were analyzed for dry matter, crude protein, neutral detergent fiber, acid detergent fiber, crude ash, calcium, phosphorus, crude fat, and gross energy (GE) according to the methods in "Feed Analysis and Feed Quality Detection Technology."Results were used to calculate nutrient apparent digestibility.

Serum antioxidant indices: Serum samples were analyzed using an L-3180 semi-automatic biochemical analyzer to determine total antioxidant capacity (T-AOC), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, and malondialdehyde (MDA) content using assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.7 Statistical Analysis

Data were initially processed using Excel 2013, then subjected to one-way ANOVA using the ANOVA procedure in SAS 9.4 statistical software. Duncan' s multiple range test was used for post-hoc comparisons, with $P<0.05$ as the criterion for significant difference.

2.1 Growth Performance

As shown in Table 4 , dietary concentrate proportion of ewes during late gestation had no significant effect on body weight of early-weaned lambs at 1, 10, 20, 30, 40, 50, and 60 days of age ($P>0.05$). Although differences in lamb body weight among groups were not significant ($P>0.05$), lambs in the 50% group had higher body weight than other groups at 1, 10, 20, 30, 50, and 60 days of age.

2.2 Body Measurements

As shown in Table 5 , dietary concentrate proportion of ewes during late gestation had no significant effect on body height, chest depth, chest circumference, abdominal circumference, head width, head length, straight crown-rump length, curved crown-rump length, or body length of early-weaned lambs at 20 and 60 days of age ($P>0.05$).

2.3 Nutrient Apparent Digestibility

As shown in Table 6 , dietary concentrate proportion of ewes during late gestation had no significant effect on apparent digestibility of dry matter, organic matter, gross energy, crude protein, crude fat, neutral detergent fiber, acid detergent fiber, calcium, phosphorus, or crude ash in early-weaned lambs ($P>0.05$).

2.4 Serum Antioxidant Indices

As shown in Table 7 , with decreasing maternal dietary concentrate proportion (lower nutrient level), serum T-AOC and SOD activity of lambs at 20 days of age increased significantly ($P<0.01$), while at 60 days of age, the 50% group was significantly or extremely significantly lower than the 40% and 30% groups ($P<0.05$ or $P<0.01$). Serum GSH-Px activity at both 20 and 60 days of age in the 30% group was significantly higher than in the 50% and 40% groups ($P<0.01$). Serum MDA content at 20 days of age decreased significantly with decreasing maternal dietary concentrate proportion ($P<0.01$), and at 60 days of age, the 50% group was significantly higher than the 30% group ($P<0.05$), while no significant difference was observed between the 40% group and the other two groups ($P>0.05$).

3.1 Growth Performance

The primary objective of ewe feeding, particularly during late gestation, is to improve the production performance of the resulting lambs. Based on the results of postnatal lamb body weight in this experiment, dietary concentrate proportion during late gestation had no significant effect on growth of early-weaned lambs. Concentrate has higher nutritional value than roughage or forage. Under conditions of identical dry matter intake, decreasing dietary concentrate proportion reduced the intake of metabolizable energy, metabolizable protein, and other available nutrients by ewes. During late gestation, the fetus is in a rapid growth and development stage, while mammary gland development and colostrum formation require additional nutrients. When maternal dietary nutrient level changes, ewes can maintain fetal development through mobilization of body reserves and improved feed nutrient utilization efficiency. Therefore, although birth weight (1-day body weight) of lambs in the 30% group was lower than in the 50% group, the difference was not significant, possibly because maternal metabolic regulation mitigated the negative effects of low dietary nutrition on fetal weight gain. Campion et al. found that when ewes were fed four energy levels (100% ME [AFRC (1993)] and 100%, 110%, 120% NE [INRA (1989)]) during late gestation, lamb birth weight did not differ significantly, but ewe body weight at parturition in the 120% NE group was significantly higher than in the 100% ME group, indicating that ewes utilized body reserves to maintain fetal development. Chadio et al. also reported that maternal nutrition level during late gestation had no significant effect on lamb birth weight, as maternal regulation maintained normal fetal development. However, Gao Feng

et al. found that when feed restriction was applied during late gestation, lamb birth weight decreased significantly with reduced feeding level. These studies suggest that when dietary nutrient levels are within the range that ewes can regulate, maternal metabolic adjustment can maintain fetal development without significantly affecting birth weight. Campion et al. also reported that late gestation nutrition had no significant effect on colostrum production or lamb colostrum intake capacity. Therefore, under early weaning conditions with identical feeding conditions and dietary nutrition for lambs, differences in ewe milk production performance had minimal impact on lamb growth, resulting in no significant differences in lamb body weight after weaning. In conclusion, dietary concentrate proportion during late gestation had no significant effect on growth of early-weaned lambs.

Although lamb body weight did not differ significantly among groups, ewe feeding costs varied. In this experiment, feeding conditions were identical during early gestation, lactation, and for lambs, so differences only existed in late gestation diet costs. Based on feed prices of 2,600 yuan/t for concentrate, 400 yuan/t for whole-plant corn silage, and 800 yuan/t for peanut straw, daily costs per ewe were: 2.97 yuan (50% group), 2.80 yuan (40% group), and 2.63 yuan (30% group). The 50% group cost 0.17 yuan more per ewe per day than the 40% group, and the 40% group cost 0.17 yuan more than the 30% group, with the 50% group costing 0.34 yuan more than the 30% group. Therefore, reducing dietary concentrate proportion did not affect early-weaned lamb growth and reduced costs, though it may affect ewe health.

3.2 Body Measurements

Body measurements are important indicators reflecting animal growth and development and evaluating body condition. Through body measurement data, we can assess growth rate, development among different body parts, and estimate live body weight in sheep. Therefore, measuring these indicators in lambs is important for understanding the effects of gestational treatments on early-weaned lamb growth and development. Throughout the experiment, no significant differences were observed in body measurements at 20 and 60 days of age, indicating that under early weaning conditions, maternal dietary concentrate proportion did not affect normal physical development of lambs. Maternal nutrition affects lamb development, and when nutrient supply is reduced during late gestation, ewes mobilize more endogenous nutrients. However, Hadiken · Kubagan et al. reported that lambs from ewes supplemented with concentrate had significantly greater body height, body length, and chest circumference at 30 days of age than non-supplemented groups. The lack of significant differences in body measurements among groups in this experiment may be due to the minimal influence of maternal milk on lamb growth under early weaning conditions. Body measurements are significantly positively correlated with body weight, and combined with the non-significant differences in lamb body weight among groups, these results indicate that under early weaning conditions, dietary con-

centrate proportion during gestation did not affect lamb body measurements.

3.3 Nutrient Apparent Digestibility

Nutrient apparent digestibility affects feed utilization efficiency and indirectly reflects animal growth performance. Digestibility is influenced not only by feed factors but also by environmental conditions and intestinal development status. The digestion trial in this experiment was conducted when lambs were 51-60 days of age. Through early weaning, lambs were under identical feeding conditions, so their nutrient digestibility was more influenced by fetal development. Cao Meng reported that when sows were fed 0.75× NRC (1998) maintenance requirement during gestation, piglets showed impaired intestinal development at birth, but normal intestinal development was restored by the end of lactation. This suggests that although maternal nutrition during gestation affects fetal intestinal development, the intestine can recover during lactation. Intestinal function and development play important roles in nutrient digestion and absorption and are crucial tissues affecting feed digestibility. Therefore, under early weaning conditions, dietary concentrate proportion during late gestation did not affect nutrient apparent digestibility in lambs.

3.4 Serum Antioxidant Indices

Serum T-AOC, SOD and GSH-Px activities, and MDA content are important indicators reflecting animal antioxidant capacity. Serum T-AOC reflects antioxidant free radical metabolism status; SOD is a natural oxygen free radical scavenger in organisms; GSH-Px protects cell membranes by clearing intracellular hydrogen peroxide and lipid free radicals; increased MDA content damages cell membrane structure and integrity as the metabolic end product of lipid peroxides. Animals clear oxygen free radicals through antioxidant enzymes. Excessive oxygen free radical generation and reduced antioxidant enzyme content can cause cellular or tissue damage. In this experiment, decreasing maternal dietary concentrate proportion improved serum antioxidant capacity in lambs, indicating that appropriately reducing concentrate proportion in maternal diet is beneficial for improving serum antioxidant capacity in early-weaned lambs. Zhang Yanyun et al. reported that energy restriction in broiler breeder hens during laying period induced compensatory growth in offspring and significantly improved serum and breast muscle antioxidant capacity. He et al. found that although energy and protein restriction during late gestation reduced serum antioxidant capacity in newborn lambs, it significantly improved serum antioxidant capacity at 6 and 22 weeks of age, with higher plasma SOD content in energy- or protein-restricted groups than in the control group. GSH-Px, SOD, and CAT are important antioxidant enzymes in the body, and their activities directly reflect antioxidant capacity; MDA is a peroxidation product, and its content indirectly reflects the degree of free radical attack. In this experiment, decreasing dietary concentrate proportion reduced maternal nutrition level, but improved serum antioxidant capacity in early-weaned lambs. The possible rea-

son is that although low supplementation groups had lower maternal nutrition levels, maternal metabolic regulation maintained fetal development, and after birth, lambs in low nutrition groups showed compensatory growth effects under identical feeding levels, leading to increased oxidative stress and improved antioxidant capacity. Tarry-Adkins et al. reported similar results when studying the effects of maternal protein restriction on antioxidant capacity in offspring skeletal muscle. Zhang Fan et al. also showed that reducing dietary concentrate proportion during late gestation improved serum antioxidant capacity in postnatal suckling lambs. In this experiment, reducing maternal dietary concentrate proportion resulted in better antioxidant performance in lambs.

Conclusion

Dietary concentrate proportion of ewes during late gestation had no significant effects on body weight, body measurements, or nutrient apparent digestibility of early-weaned lambs, but decreasing maternal dietary concentrate proportion improved serum antioxidant capacity in lambs.

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Note: Figure translations are in progress. See original paper for figures.