# Effects of Dietary Nutritional Level in Late Gestation Ewes on Growth Performance, Organ Development, and Serum Antioxidant Indices of Postnatal Lambs (Postprint)

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## Abstract

This experiment was conducted under consistent dry matter intake conditions by altering the concentrate supplement to forage ratio to regulate the dietary nutrient level of ewes during late gestation, aiming to investigate its effects on growth performance, organ development, and serum antioxidant indices of postpartum lambs. Sixty-six healthy primiparous Hu sheep ewes carrying twins at 90 days of gestation with similar age [(11.0$±1.0)*months*]*andbodyweight*[(44.43±2.20)*kg*]*wereselectedanddividedinto*3*groupswith*22*ewespergroup*
5(5 : 5*group*), 4 : 6(4 : 6*group*), *and*3 : 7(3 : 7*group*)*untillambing*; *afterlambing*, *ewesinallgroupswerefedthesa*
1)*Thedietarynutrientlevelofewesduringlategestationhadnosignificanteffectonlambbirthweightorlambbody*
0.05); *however*, *withdecreasingnutrientlevel*, *therewasatendencyforreducedlambbirthweight*(0.05 *P* <
0.10)*andincreasedbodyweightat*40*and*50*daysofage*(0.05 $P<0.10). 2) At birth (0 days), with decreasing dietary nutrient level of ewes during late gestation, chest depth of lambs increased extremely significantly (P<0.01), while body length decreased significantly (P<0.05); at 60 days of age, with decreasing dietary nutrient level of ewes during late gestation, chest depth and crown-rump length of lambs increased significantly or extremely significantly (P<0.05 or P<0.01). 3) At 10 days of age, the weights of liver and lungs of lambs decreased significantly with decreasing dietary nutrient level of ewes during late gestation (P<0.05); at 60 days of age, there were no significant differences in tissue and organ weights among groups (P>0.05). 4) At 20 and 60 days of age, with decreasing dietary nutrient level of ewes during late gestation, total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, and glutathione peroxidase (GSH-Px) content in lamb serum increased significantly or extremely significantly (P<0.05 or P<0.01), while malondialdehyde (MDA) content decreased extremely significantly (P<0.01). When regulating the dietary nutrient level of ewes through the concentrate supplement to forage

ratio during late gestation, it was found that low nutrient level (low concentrate supplement proportion) could reduce lamb birth weight, but through compensatory growth, the serum antioxidant capacity of lambs was improved and growth performance was restored.

## Full Text

## Effects of Maternal Dietary Nutrient Level in Late Gestation on Growth Performance, Organ Development, and Serum Antioxidant Capacity of Postpartum Lambs

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**Abstract:** This experiment investigated the effects of altering the concentrate supplement to forage ratio to regulate dietary nutrient levels in late gestation ewes on the growth performance, organ development, and serum antioxidant capacity of postpartum lambs, while maintaining consistent dry matter intake. Sixty-six healthy primiparous Hu ewes at 90 days of gestation with similar age [(11.0$±1.0)monthsandbodyweight[(44.43±2.20)kg]wererandomlydividedintothreegroups(n = 22).Eweswerefedconcentratesupplementandforageatdrymatterratiosof5 : 5(5 : 5group), 4 : 6(4 : 6group), and3 : 7(3 : 7group)untilparturition.Afterlambing, allewesreceivedthesameto
(1)Maternaldietarynutrientlevelduringlategestationhadnosignificanteffectonlambbirthweightorbodyweigh
0.05).However, withdecreasingnutrientlevels, birthweightshowedadecreasingtrend(0.05 P < 0.10), whilebodyweightat40and50daysshowedanincreasingtrend(0.05 $P<0.10). (2) At birth, decreasing maternal dietary nutrient level significantly increased chest depth (P<0.01) and decreased body length (P<0.05) of lambs. At 60 days, decreasing maternal nutrient level significantly or extremely significantly increased chest depth and curved crown-rump length (P<0.05 or P<0.01). (3) At 10 days, liver and lung weights decreased significantly with decreasing maternal dietary nutrient level (P<0.05). At 60 days, no significant differences were observed in organ weights among groups (P>0.05). (4) At 20 and 60 days, decreasing maternal dietary nutrient level significantly or extremely significantly increased serum total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, and glutathione peroxidase (GSH-Px) content (P<0.05 or P<0.01), while extremely significantly decreasing malondialdehyde (MDA) content (P<0.01). These findings indicate that low nutrient levels (lower concentrate supplement ratio) during late gestation may reduce lamb birth weight, but through compensatory growth, lambs exhibit improved serum antioxidant capacity and restored growth performance.

**Keywords:** late gestation; nutrient level; Hu sheep; lamb; body weight; organ development; antioxidant capacity

Late gestation is a critical period for embryonic development in ewes, during which maternal nutrient requirements increase. Nutritional restriction during this period can affect offspring organ development, growth performance, and reproductive performance [1]. Hu sheep, a renowned prolific breed, are characterized by rapid growth, strong reproductive capacity, suitability for intensive feeding, and excellent meat production and quality [2], making them widely promoted in the mutton sheep industry. In ruminant feeding, dietary nutrient levels can be increased by either raising the nutritional value of concentrate supplements or increasing their feeding amount [3]. Therefore, studying the effects of regulating dietary nutrient levels in late gestation by adjusting the concentrate supplement to forage ratio on postpartum lamb development has important practical significance. Previous research has examined the effects of dietary concentrate-to-forage ratios on animal production performance. Cui et al. [4] found that increasing concentrate supplement levels reduced ewe weight loss during parturition and increased lamb birth weight, with a 5:5 concentrate-to-forage ratio showing the best economic benefit. He et al. [5] reported that energy and protein restriction in late gestation decreased antioxidant capacity in offspring lambs, but most antioxidant indexes and gene expression levels recovered to normal after postpartum nutritional restoration. Van Emon et al. [6] found that protein level in late gestation had no significant effect on lamb birth weight, while weaning weight and daily gain showed increasing trends with higher protein levels. Thus, dietary concentrate-to-forage ratio affects feed utilization efficiency and ruminant production performance [7], and maternal nutrition during gestation influences offspring health.

Although studies have investigated the effects of dietary concentrate-to-forage ratios on animal growth and metabolism, as well as the impacts of energy and protein levels during late gestation on postpartum lamb development, research on regulating dietary nutrient levels through concentrate supplement to forage ratio manipulation in late gestation ewes remains limited. This experiment, conducted under consistent dry matter intake conditions, altered the concentrate supplement to forage ratio to regulate dietary nutrient levels in late gestation ewes, aiming to investigate the effects on postpartum lamb growth performance, organ development, and serum antioxidant indexes. The findings provide a theoretical basis for understanding how maternal dietary nutrient levels during late gestation affect offspring health and offer guidance for diet formulation in gestating ewes under intensive feeding conditions.

### 1.1 Experimental Animals and Design

This experiment was conducted from July 31, 2015, to February 28, 2016, at Linqing Runlin Animal Husbandry Co., Ltd. in Shandong Province. One hundred forty healthy primiparous Hu ewes with similar body condition, age, and weight were artificially inseminated with semen from the same ram. During days 1–90 of gestation, all ewes received the same total mixed ration (TMR) ad libitum. After pregnancy diagnosis by ultrasound on day 50, 66 twin-bearing ewes were selected and randomly divided into three groups (n=22) on day 90 of gestation [body weight (44.43$±2.20)*kg*, (11.0±$1.0) months of age] based on consistent body weight. From day 90 of gestation to parturition, each group received the same concentrate supplement and forage but in different ratios (5:5, 4:6, and 3:7 on a dry matter basis) through quantitative feeding. After lambing, all ewes were fed the same TMR ad libitum.

One hundred thirty-two lambs were raised with their mothers until 10 days of age, and three male lambs from each group (selected from the 44 lambs per group) were slaughtered for tissue collection. One lamb per ewe (n=22 per group) was raised with its mother until weaning at 60 days of age, then slaughtered for measurements.

### 1.2 Experimental Diets

All groups received the same concentrate supplement and forage but in different ratios (5:5, 4:6, and 3:7). The nutrient level for the 5:5 group diet was formulated according to the nutritional requirements for late gestation ewes established by our research group [8]. The lowest dry matter intake group (3:7 group) served as the baseline feeding level for all groups. Daily concentrate supplement amounts per ewe were 720 g, 570 g, and 420 g (air-dry basis), while forage amounts were 2.28 kg, 2.74 kg, and 3.20 kg (wet weight) for the 5:5, 4:6, and 3:7 groups, respectively, ensuring complete consumption. The concentrate supplement was pelleted, and the forage consisted of whole-plant corn silage and peanut seedlings mixed at a 1:1 dry matter ratio. Diet composition and nutrient levels are presented in Table 1 . The premix was a 4% gestating ewe premix from Beijing Precision Animal Nutrition Research Center. Nutrient levels were determined according to *Feed Analysis and Feed Quality Detection Technology* [9].

### 1.3 Animal Management

Before the experiment, all pens were cleaned and disinfected, all animals underwent quarantine and deworming, and pens and feed troughs were separated for feeding two ewes per pen. Water and mineral blocks were provided ad libitum, and pens were disinfected regularly on Mondays and Thursdays. During gestation, ewes were fed twice daily at 06:30 and 16:00, with equal amounts at each feeding. Forage was offered first and consumed completely before concentrate supplement was provided.

### 1.4 Measurements

**1.4.1 Lamb Body Weight** Lamb body weight was measured at 0, 10, 20, 30, 40, 50, and 60 days after birth before 08:00.

**1.4.2 Lamb Body Measurements** Body measurements were taken at 0 and 60 days after birth. Measurements included: body height (vertical distance from the highest point of the scapula to the ground), chest depth (vertical distance from the withers to the lower edge of the sternum), chest circumference (circumference around the chest behind the scapula), abdominal circumference (vertical circumference at the largest abdominal point), head width (distance between lateral temporal muscle lines), head length (vertical distance from head top to chin), straight crown-rump length (vertical distance from forehead to tail vertebra end), curved crown-rump length (distance from forehead along the back to tail vertebra end), and body length (distance from shoulder end to ischial tuberosity end).

**1.4.3 Tissue and Organ Development** At 10 and 60 days after birth, three male lambs with body weight close to the group average were selected from each group for slaughter. Weights of the heart, liver, spleen, lung, kidney, stomach, and small intestine were recorded.

**1.4.4 Blood Collection and Antioxidant Index Determination** At 20 and 60 days after birth, eight lambs per group (half male and half female) were selected for jugular vein blood collection. Blood samples were centrifuged at 3,000 r/min for 15 minutes, and serum was collected in 1.5 mL tubes and stored at -20 °C for antioxidant analysis. Serum total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-Px) content, and malondialdehyde (MDA) content were measured using an L-3180 semi-automatic biochemical analyzer with assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

### 1.5 Data Processing and Statistical Analysis

Data were initially processed using Excel 2013 and then analyzed using one-way ANOVA in SAS 9.4. Duncan' s multiple comparison test was used for post-hoc analysis. The significance trend was defined as $0.05 \leq P < 0.10$, significance as $P<0.05$, and extreme significance as $P<0.01$.

### 2.1 Effects of Maternal Dietary Nutrient Level on Postpartum Lamb Growth Performance

The effects of maternal dietary nutrient level during late gestation on postpartum lamb growth performance are shown in Table 2 . Altering the concentrate supplement to forage ratio to regulate dietary nutrient level had no significant effect on lamb body weight at any time point ($P>0.05$). However, with decreasing maternal dietary nutrient level, lamb birth weight (0 day) showed a decreasing trend ($0.05 \leq P < 0.10$), while body weight at 40 and 50 days showed an increasing trend ($0.05 \leq P < 0.10$). The average daily gain of lambs in the 3:7 group was higher than the other two groups, but the difference was not significant ($P>0.05$).

### 2.2 Effects of Maternal Dietary Nutrient Level on Postpartum Lamb Body Measurements

The effects of maternal dietary nutrient level during late gestation on postpartum lamb body measurements are presented in Table 3 . At birth, maternal dietary nutrient level had no significant effect on body height, chest circumference, abdominal circumference, head width, head length, or curved crown-rump length ($P>0.05$). The chest depth of lambs in the 3:7 group was extremely significantly higher than in the 5:5 and 4:6 groups ($P<0.01$). With decreasing maternal dietary nutrient level, straight crown-rump length showed a decreasing trend ($0.05\leq P<0.10$), and body length in the 3:7 group was significantly lower than in the 5:5 and 4:6 groups ($P<0.05$). At 60 days, no significant differences were observed among groups in body height, chest circumference, abdominal circumference, head width, or head length ($P>0.05$). Chest depth in the 3:7 group was significantly higher than in the 4:6 group ($P<0.05$). With decreasing maternal dietary nutrient level, straight crown-rump length showed an increasing trend ($0.05\leq P<0.10$), and curved crown-rump length in the 3:7 group was extremely significantly higher than in the 5:5 and 4:6 groups ($P<0.01$).

### 2.3 Effects of Maternal Dietary Nutrient Level on Postpartum Lamb Tissue and Organ Development

The effects of maternal dietary nutrient level during late gestation on postpartum lamb tissue and organ development are shown in Table 4 . At 10 days, liver and lung weights decreased significantly with decreasing maternal dietary nutrient level ($P<0.05$), while no significant effects were observed on heart, spleen, kidney, stomach, or small intestine weights ($P>0.05$). At 60 days, maternal dietary nutrient level had no significant effect on the weights of heart, liver, spleen, lung, kidney, stomach, or small intestine ($P>0.05$).

### 2.4 Effects of Maternal Dietary Nutrient Level on Postpartum Lamb Serum Antioxidant Indexes

The effects of maternal dietary nutrient level during late gestation on postpartum lamb serum antioxidant indexes are presented in Table 5 . At 20 days, serum T-AOC in the 4:6 and 3:7 groups was extremely significantly higher than in the 5:5 group ($P<0.01$). Serum SOD activity and GSH-Px content in the 3:7 group were significantly higher than in the 5:5 group ($P<0.05$), while serum MDA content in the 5:5 group was extremely significantly higher than in the 4:6 and 3:7 groups ($P<0.01$). At 60 days, with decreasing maternal dietary nutrient level, serum T-AOC, SOD activity, and GSH-Px content increased extremely significantly ($P<0.01$), while MDA content decreased extremely significantly ($P<0.01$).

### 3.1 Effects of Maternal Dietary Nutrient Level on Postpartum Lamb Growth Performance

Ruminants have lower digestibility and utilization efficiency for forage than for concentrate supplements. Therefore, when the forage proportion increases and concentrate supplement proportion decreases, the intake of metabolizable energy, metabolizable protein, and other nutrients decreases accordingly. During late gestation, the embryo grows rapidly. When maternal dietary nutrient levels cannot meet the demands of normal embryonic development, ewes mobilize their own nutrient reserves [11] to maximize fetal nutrient supply, often at the cost of reduced maternal weight gain or even weight loss [12]. Chadio et al. [13] reported that maternal nutrient level during late gestation had no significant effect on lamb birth weight, as maternal adaptive regulation maintained healthy embryonic development. Van Emon et al. [14] found that metabolizable protein level in late gestation had no significant effect on lamb birth weight or weaning weight. In this study, no significant differences were observed in lamb birth weight, consistent with Wang et al. [15], who reported no significant differences in birth weight when ewes were fed 0.8, 0.9, and 1.0 times the NRC (2007) nutrient requirements during late gestation. However, maternal body weight was significantly affected, indicating that this nutritional level was within the tolerance range of ewes, which sacrificed body weight to maintain fetal growth. The decreasing trend in birth weight suggests that maternal nutrition does affect embryonic development to some extent. When the maternal nutritional regulatory buffer system is disrupted, fetal development and maternal health are severely compromised [16]. Gao et al. [17] reported that feed restriction during late gestation significantly affected lamb birth weight, but differences disappeared by 28 weeks of age. In this experiment, maternal nutritional regulation maintained lamb birth weight, and postpartum lambs developed normally due to adequate maternal nutrition and milk intake. Although body weight differences were not significant, lambs in the 3:7 group showed increasing trends in body weight at 40 and 50 days and higher average daily gain, indicating that maternal nutritional restriction during gestation promoted postnatal lamb growth under adequate nutrition. Previous studies have reported that offspring from nutrient-restricted pregnant mice exhibited stronger feeding ability and faster growth when provided with adequate postnatal nutrition [18]. Gao et al. [19] studied compensatory growth in feed-restricted ewes during late gestation and found that restricted groups produced heavier lambs after compensatory growth. Our results are similar, showing that low maternal nutrient levels during gestation increased postnatal feed intake and promoted growth through compensatory growth mechanisms.

### 3.2 Effects of Maternal Dietary Nutrient Level on Postpartum Lamb Body Measurements

Body measurements reflect growth and development status, serving as important indicators of body size, structure, and development, and indirectly reflecting organ development [20]. While body weight indicates overall growth, it cannot reflect specific body part development, making body measurements crucial for evaluating lamb development. At birth, decreasing maternal dietary nutrient level increased chest depth and circumference but decreased straight crown-rump length and body length, indicating that maternal nutrition affected body measurements despite no significant effect on body weight. Decreasing concentrate supplement proportion and increasing forage proportion reduced body length but increased chest circumference. At 60 days, compensatory growth resulted in increased straight crown-rump length, curved crown-rump length, and body length with decreasing maternal nutrient level, indicating improved growth rate. Gao et al. [20] reported compensatory growth responses in fetal-growth-restricted Sunit sheep. Compensatory growth is the phenomenon of accelerated growth when nutrition is restored after previous restriction, with five levels: over-compensation, full compensation, partial compensation, zero compensation, and negative compensation [21]. The superior body measurements in the low nutrient level group at 60 days suggest that lambs may have been in an over-compensatory growth state through increased feed intake.

### 3.3 Effects of Maternal Dietary Nutrient Level on Postpartum Lamb Tissue and Organ Development

Adaptive changes in the intrauterine environment have decisive effects on fetal and neonatal health [22], and nutrient intake levels, diet quality, and quantity affect visceral organ metabolism and development [23]. This study found that decreasing maternal nutrient level during late gestation significantly reduced liver and lung weights in 10-day-old lambs. The liver is a vital metabolic organ, and maternal nutrition during gestation affects its development. Nutritional restriction may limit placental blood flow, restricting fetal oxygen and nutrient supply and affecting lung development. Under consistent dry matter intake, different concentrate supplement to forage ratios affected nutrient intake levels, and nutrient restriction influenced blood oxygen supply and lung development. Gao et al. [17] also reported that gestational nutrient restriction significantly reduced liver and lung weights at birth, but differences disappeared by 28 weeks. Although other organ weights were not significantly affected, they tended to decrease with decreasing concentrate supplement level, indicating that maternal nutrient level during late gestation influences organ development. He et al. [24] reported that 40% energy or protein restriction during late gestation altered fetal growth and visceral organ mass, but most organ weights recovered by 6 weeks postpartum. In this study, the lack of significant differences in organ weights at 60 days may be attributed to compensatory growth during the suckling period, which mitigated the effects of gestational nutrient restriction. However, weight alone does not fully reflect structural and functional recovery, and organ function may still be affected despite similar weights.

### 3.4 Effects of Maternal Dietary Nutrient Level on Postpartum Lamb Serum Antioxidant Capacity

Animals continuously produce various antioxidant substances to eliminate excess free radicals and maintain homeostasis, protecting cells from oxidative damage. Serum T-AOC, SOD activity, GSH-Px content, and MDA content reflect antioxidant capacity. T-AOC is a key indicator of overall antioxidant capacity, reflecting the metabolic status of the antioxidant defense system [25]. SOD is a natural scavenger of oxygen free radicals, and its activity reflects the ability to eliminate them [26]. GSH-Px removes hydrogen peroxide and lipid free radicals, protecting cell membranes [27]. MDA is the end product of lipid peroxidation that oxidizes unsaturated fatty acids and damages cell membrane structure and integrity [28], while also harming the antioxidant defense system. Zhang et al. [29] found that maternal nutrient restriction during gestation significantly reduced embryonic serum T-AOC, SOD activity, and GSH-Px content while increasing MDA content. In contrast, this study found that serum T-AOC, SOD activity, and GSH-Px content increased significantly or extremely significantly with decreasing maternal nutrient level at 20 and 40 days, while MDA content decreased extremely significantly. This discrepancy may be due to the excessively low nutrient level in Zhang et al. [29], which disrupted maternal nutritional balance and affected embryonic development, preventing adequate compensatory recovery. Zhang et al. [30] reported that energy restriction in broiler breeder hens during late lay improved offspring antioxidant capacity during compensatory growth. Pan et al. [31] also found that early feed restriction significantly increased serum SOD content in broilers after compensatory growth. He et al. [5] reported that energy and protein restriction during gestation reduced plasma antioxidant capacity at birth, but SOD content and related gene expression in liver and thymus were higher in restricted groups at 6 and 22 weeks postpartum. Our results align with these studies, showing that low maternal nutrient levels during late gestation improved offspring serum antioxidant capacity. This may be because maternal nutrition was within a controllable regulatory range, and lambs exhibited compensatory growth with stronger antioxidant capacity under adequate milk supply during the suckling period. These results suggest that low nutrient diets during late gestation are beneficial for improving postpartum lamb antioxidant capacity when maternal nutritional regulation capacity is not overwhelmed and adequate milk supply is available.

In conclusion, regulating maternal dietary nutrient level during late gestation through concentrate supplement to forage ratio manipulation revealed that low nutrient levels (lower concentrate supplement proportion) tended to reduce lamb birth weight but improved serum antioxidant capacity and restored growth performance through compensatory growth.

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