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Article

Impact of early weaning and nutritional interventions on growth performance, digestive metabolism, and serum biomarkers in lambs: A biomechanical perspective

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Abstract: The aim of this study was to investigate the effects of early weaning and nutritional interventions on growth performance, nutrient digestion and metabolism, and serological indices of hu sheep lambs, with a view to providing a basis for the feasibility of early weaning of hu sheep lambs under conditions of supplemental milk replacer. Ninety neonatal hu sheep lambs were weaned at 21 days (n = 60) (divided into two groups: early weaned, EW; and resveratrol-fed, RSV) as well as 49 days (n = 30) (control group, CON) weaning, and the trial period was 90 days. The results showed that: 1) there was no significant difference ($P > 0.05$) in the initial and final weights of the lambs, and the average daily gain (ADG) of the EW and RSV groups was significantly lower than that of the CON group ($P < 0.05$) from 31 to 45 days of age. Early weaned lambs were more susceptible to weaning stress compared to late weaned lambs and the effect of nutritional intervention (feeding resveratrol) on lamb growth performance was not significant. 2) The apparent digestibility of crude protein (CP) in the experimental group was significantly lower than that of the control group ($P < 0.05$), and the nitrogen intake, net protein utilization and protein biological value indexes of lake lambs in the experimental group were significantly different from those of the control group ($P < 0.05$). 3) Early feeding of resveratrol was not significant in improving the digestive metabolism of nutrients. 4) Early weaning as well as the addition of resveratrol had a significant effect on the serum GLU and TC indexes of the lambs ($P < 0.05$), but did not show a significant effect on any of the other indexes.

Keywords: lambs; early weaning; growth performance; digestive metabolism; serum indices; biomechanical; biomarkers; molecular

1. Introduction

Weaning is a critical stage in the growth of lambs. Early weaning shortens the reproductive cycle of ewes, improves flock productivity by increasing lambing frequency, and can largely reduce production costs [1], and has therefore become a common practice in modern sheep farming to improve overall efficiency. However, the replacement of breast milk with plant-based feeds, change in feeding regime and separation from ewes during weaning is tremendously stressful for the lambs and has the potential to adversely affect the growth, development and health of the lambs, which exhibit weight loss, increased morbidity and mortality during the weaning period [2].

Hu sheep is one of the important sheep breeds in the Taihu Lake basin of China, which has the advantages of high reproductive rate, long estrus period, high average

lambing rate, fast growth rate, strong environmental adaptability, good lactation performance, and fast growth and development [3]. Therefore, the present study was conducted to investigate the effects of early weaning stress on the growth performance and blood indexes of Hu sheep lambs with high reproduction rate and wide rearing range, and to further analyze whether nutritional interventions after early weaning would alleviate the negative effects of weaning stress on the growth performance and blood indexes of lambs. We also analyzed whether the nutritional intervention after early weaning would reduce the negative effects of early weaning stress on the growth performance and blood indexes of lambs.

2. Literature review

Weaning is a very critical part of the lamb breeding process. Early weaning (around 30 days or even earlier) can maximize the use of ewes relative to conventional weaning (45–60 days), and is conducive to the rapid growth and development of lambs and the early release of lambs, maximizing the benefits [4]. Weaning stress is a hotspot of nutritional physiological research on young animals in recent years, which mainly includes two aspects of psychological stress and nutritional stress. Nutritional stress mainly refers to the huge shift in feed structure, where structural carbohydrates replace lactose and milk fat as the main energy source, and the digestive system and physiology of lambs need to adapt to this shift; and the change in nutrient intake affects the metabolism and other physiological processes of lambs. Moreover, the immune system of newborns is not fully established, and passive immunity from the mother provides early protection for young animals, while nutrient intake is critical for the establishment of the immune system and the maintenance of a balance between immune regulation and immune response. Studies have shown that the effects caused by weaning stress appear 1 to 3 days after weaning and have long-term effects [5].

Determination of weaning time is an important part of lamb feeding management. Weaning can lead to psychological and physiological stress in lambs due to the severance of the mother-child relationship and may even adversely affect lamb growth [2]. Studies have shown that weaning has both short and long term effects on the functional response of the adrenal cortex in lambs, with a significant increase in serum cortisol concentration and a dramatic increase in respiratory rate in lambs after weaning [6]. Weaning stimulation leads to behavioral changes in young animals such as shouting, restlessness, frequent walking, and reduced feed intake, which in severe cases can adversely affect normal growth and development [7]. Belanche et al. [8] found that lambs lactating with their mothers had a better-developed immune system when compared to artificially fed ewes with colostrum or colostrum substitutes, which has a negative impact on the better advantage of their later growth performance.

Studies have shown that early weaned lambs, despite the significant increase in open feed intake, due to the lack of breast milk and at this time the lambs' ability to digest and utilize plant-based feeds is weaker, which is prone to induce diarrhea and other problems, resulting in the growth and development of lambs being impeded [9]. Animals in the young stage are in the period of the fastest growth and development of the organism, rapid improvement of tissue and organ functions, accelerated construction of the immune system, and great plasticity. Weaning can affect the

growth and health of young ruminants, and by taking some necessary measures, such as nutritional regulation through feeding management, dietary supply and feed additives, it can alleviate the weaning stress of lambs and reduce the potential harm caused by stress to young ruminants [10]. Bhatt et al. [11] found that pre-weaning nutritional intervention had a significant effect on the growth performance of lambs after weaning, and the pre-weaning weight gain of lambs was controlled by feeding milk replacer to minimize the age at weaning.

Functional plant compounds can be used to enhance the immune system in humans, thus helping to control disease and inflammation [12]. In recent years, functional plant compounds have been used as livestock and poultry feed additives and offer many advantages such as immunomodulatory properties, reduced disease incidence, increased feed intake, and growth promotion [13]. With the banning of antibiotics, the development of alternative strategies has attracted the attention of many researchers. Among them, resveratrol has been extensively studied in livestock and poultry. The study of Cao et al. [14] showed that resveratrol (additive dose of 100 mg/kg) could effectively alleviate intestinal damage caused by oxidative stress injury in piglets, which could enhance the epithelial barrier function, improve the redox state, attenuate mitochondrial damage and induce mitochondrial phagocytosis. Resveratrol has an effect on immune function in piglets. Fu et al. [15] showed that resveratrol can be used as an adjuvant to enhance immune response to vaccines, and it can also be considered as a feed additive to enhance humoral and cellular immunity. Studies in aquatic animals have shown that resveratrol has some antioxidant and lipid metabolism regulating effects, but may have negative effects affecting feed intake and growth performance and is less recommended as an aquatic feed additive [16].

In summary, resveratrol as livestock and poultry feed addition to a certain extent has the effect of improving animal production performance, inhibiting oxidative stress and inflammatory response etc. However, at present, there is no report on whether it has a certain effect on alleviating weaning stress, and whether resveratrol has an effect on the growth performance and blood indexes of young ruminants after weaning has not been reported in the research on lambs, which needs to be further researched.

3. Materials and methods

The experimental procedures complied with the requirements of the CAAS Animal Ethics Committee, and the investigators strictly followed humane animal care and handling procedures throughout the experiment. This study was conducted from July to October 2023 at the sheep farm of Ningxia Nongken Helanshan Beef and Sheep Industry Group.

3.1. Experimental design

From the newborn lambs, 90 lambs were selected from natural births, with a difference of no more than 3 d in date of birth, a birth weight close to (3.82 ± 0.46) kg, in good condition, healthy and free of disease, and the difference in body weight between the groups was not significant ($P > 0.05$). The lambs were randomly divided into 3 groups with 3 replicates of 10 lambs each, and the grouping ensured that the lambs in each group had similar body weights, as well as a comparable number of

individual male and female lambs in each group. The 3 groups were the control group (CON group, 45 d weaning), the early weaning group (EW group, 21 d weaning), and the resveratrol group (RSV group, fed with 10 mg/kg of BW resveratrol, and weaned for 21 d), respectively.

All three groups of lambs were fed with breast milk + supplemental milk replacer. Lambs in the EW and RSV groups were weaned at 21 d, and lambs in the control group were suckled by their mothers until 45 d. All lambs were exclusively breastfed for the first 8 d of life. Supplementary feeding of milk replacer was started from 8 d to 75 d and fattening feed was fed from 65 d. The RSV group was fed additives at 7:00 am every morning starting on the 4 days of age. The sources and feeding doses of additives were as follows: resveratrol was purchased from Hunan Mecoda Bioresources Co. Ltd, extracted from tiger cane, the product was white powdery solid with purity > 98%. The amount of additive per lamb was 10 mg/kg BW. Resveratrol was diluted with glucose in the ratio of 1:5 by weight, and the specific feeding dose was: 300 mg/d (containing 50 mg of resveratrol) per lamb during 4–35 d; 600 mg/d (containing 100 mg of resveratrol) per lamb during 36–75 d. The additive was also used as a dietary supplement for the lambs, and the dosage was as follows: the additive was purchased from Hunan Mecoda Bioresources Co.

3.2. Weaning methods and feeding management

The experimental site was a semi-open barn with good lighting and ventilation. All test lambs were ear-numbered 3 d after birth and immunized according to the farm's vaccination program. After birth, the lambs were nursed with their mothers in the same pen, and the lambs in the EW and RSV groups were separated from their mothers at 21 d (the lambs stayed in place and the mothers were removed) and weaned at once; the lambs in the CON group continued to be nursed with their mothers at 21 d, and then separated from their mothers at 45 d (the lambs stayed in place and the mothers were removed) and weaned at once. All lambs were supplemented with milk replacer from 8 d, once a day at 07:00 and once a day at 19:00, and the time spent with the ewes was gradually reduced, and the time spent on supplementation was increased by 0.5~1.0 h per day, and the intake of breast milk was gradually reduced until the lambs were completely weaned at 21 and 45 days of age. The milk replacer was provided by a Ningxia Research Institute of Pastoral Science and Technology, and the nutritional level of milk replacer is shown in **Table 1**. Fattening feed were prepared by Ningxia Nongken Cattle and Sheep Industry Group (Co., Ltd.), and the main raw materials were alfalfa, silage, concentrates, soybean meal, and corn, etc., and the composition of the fattening feed and their nutritional levels are shown in **Table 2**.

Three groups of lambs were fed milk replacer + fattening feed from 65 days of age, and the lambs were allowed to transition gradually, and at 75 days of age, they were transitioned to direct feeding of fattening feed until 90 days of age. **Table 3** shows the feeding methods at different ages of Hu sheep lambs, and the actual daily ration intake and the behavior of the lambs in each group were recorded in detail during the trial. The lambs were given clean water and no antibiotics were used throughout the trial.

Table 1. Nutrient levels of milk replacer (DM basis) %.

Items	Content
Dry matter (DM)	85.57
Crude protein (CP)	17.06
Ether extract (EE)	4.54
Neutral detergent fibres (NDF)	63.71
Acid detergent fiber (ADF)	10.95
ASH	8.55
Calcium (CA)	0.89
Total phosphorus (TP)	0.32
Metabolic energy (ME/(MJ/kg))	10.20
Digestive energy (DE/(MJ/kg))	12.55

Note: Nutrient levels were measured values except for ME and DE.

Table 2. Composition and nutrient levels of fattening feed (DM basis).

Ingredients	Content	Nutrient levels	Content (%)
Corn silage	27.03	DM	64.84
Soybean residue	21.62	CP	15.78
Corn	17.84	EE	2.58
Alfalfa	10.81	NDF	67.29
Concentrate feed	9.73	ADF	26.06
Maize straw	8.11	ASH	11.80
Premix	2.60	CA	0.63
Soybean meal	1.62	TP	0.28
NaCL	0.32	ME/(MJ/kg)	9.85
Na_2CO_2	0.32	DE/(MJ/kg)	12.08
Total	100.00		

1) One kg concentrate feed contained: CP250g, CF150g, Ca 40g, TP 0.5g, Ash190g, NaCl 40g.
2) One kg premix contained: VA 300 000 IU, VD 100 000 IU, VE 2 100 IU, Mg 600 mg, Mn950 mg, Zn 1 800 mg, Se 12 mg, Co 15 mg.
3) Nutrient levels were measured values except for ME and DE.

Table 3. Feeding mode of Hu sheep lambs at different ages.

Days of age	CON group	EW group	RSV group
1~3	breast milk	breast milk	breast milk
4~7	breast milk	breast milk	breast milk + RSV
8~21	breast milk + milk replacer	breast milk + milk replacer	breast milk + milk replacer + RSV
22~45	breast milk + milk replacer	milk replacer	milk replacer + RSV
46~65	milk replacer	milk replacer	milk replacer + RSV
66~75	milk replacer + fattening feed	milk replacer + fattening feed	milk replacer + fattening feed + RSV
76~90	fattening feed	fattening feed	fattening feed + RSV

3.3. Measurement indicators and methods

3.3.1. Lamb growth performance indicators

All lambs were weighed before morning feeding at 1, 15, 30, 45, 60, 75, and 90 days of age, and ADG was calculated for each stage. At the same time, starting from 8 days of age, the amount of open-feed intake was recorded daily for each pen of lambs.

Average daily feed intake = (feed intake − feed leftover)/number of days in the experiment;

Feed to weight ratio = ration consumption during the feeding period (kg)/weight gain during the same period (kg).

3.3.2. Measurement of digestive and metabolic indicators in lambs

At 50 days of age, 6 lambs from each group close to the average weight of the group were randomly selected for the total collection of feces and urine method. The digestive metabolism test was conducted in specially designed digestive metabolism cages, where feces could be collected centrally and urine could be discharged directly through a pipe to a designated collection container. The test period was 12 d, including 7 d of pre-testing (to fully acclimatize the lambs to the cages to reduce stress and test errors) and 5 d of formal testing. During this period, the open diet was kept unchanged and water was freely available. Feces and urine collection was carried out for 24 h at the beginning of the positive test period. DM, OM, CP, EE, Ash, Ca and P were determined by the method of AOAC [17].

The relevant formulae are as follows：

Apparent digestibility of nutrients (%) = 100 × (nutrient content of ingested nutrients − nutrient content of feces)/nutrient content of ingested nutrients.

Total excreted nitrogen (g/d) = fecal nitrogen + urinary nitrogen.

Deposited nitrogen (g/d) = ingested nitrogen − (fecal nitrogen + urinary nitrogen).

Net protein utilization (%) = 100 × deposited nitrogen/fed nitrogen.

3.3.3. Measurement of serum biochemical indices in lambs

Ten test lambs in each group were selected on the day of weaning, on the 5th day after weaning (i.e., 26, 26, and 50 days of age), and on the 90th day of age, and 10 mL of blood was drawn from the jugular vein on an empty stomach, and the serum was collected by centrifugation (3000 r/min, 15 min) at the scene and placed in a refrigerator at −20℃ for measurement. Serum levels of glucose (GLU), total protein (TP), albumin (ALB), globulin (GLB), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglycerides (TG) were measured. As well as measuring the activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP). The nutritional status of the lambs was reflected by the above indexes. The above indexes were determined by using a reagent kit and biochemistry instrument (Beijing Meirisheng Pharmaceutical Technology Co., Ltd.), and the time of blood collection was as shown in **Table 4**.

Table 4. Blood sampling time.

Groups	21	26	45	50	90
Control group			√	√	√
Early weaning group	√	√			√
Resveratrol group	√	√			√

√ means that blood collection of Hu sheep lambs is carried out at this age.

3.3.4. Data processing

All data were analyzed using SAS 9.2 software (SAS Inst. Inc., Cary, NC) after preliminary organization in Excel 2010. The data were analyzed using one-way ANOVA model and multiple comparisons were tested using Duncan's method.BW, ADG, nutrient apparent digestibility, and serum indexes were analyzed on a per-sheep test unit basis, and feed intake and feed conversion ratio were analyzed on a per-replicate test unit basis. $P < 0.05$ indicates a significant difference, $P < 0.01$ indicates a highly significant difference, and $P \geq 0.05$ indicates a difference were not significant.

4. Results

4.1. Effects of early weaning and nutritional interventions on lamb growth performance

For the 90 lambs in this trial, a total of 87 lambs were included in the experimental data statistics, except for 2 lambs eliminated from the CON group due to ewe illness and 1 accidentally dead lamb. As shown in **Table 5**, there was no significant difference ($P > 0.05$) in the initial (1 d) weights of the lambs in the three groups, indicating that the selected lambs were of the same weight and that the initial weights were in accordance with the principle of randomized grouping. By comparing the initial and final weights of the lambs it can be seen that there was no significant difference ($P > 0.05$). After early weaning, the weight of lambs in the EW and RSV groups were significantly lower than that of the CON group ($P < 0.05$), but there was no significant difference between the weights of lambs in the EW and RSV groups ($P > 0.05$). From 31 to 45 days of age, the ADG of the EW group was significantly lower than that of the CON group ($P < 0.05$), and the ADG of the CON was also much higher than that of the RSV group, while the differences among the three groups were not significant ($P > 0.05$) at other time periods. The average daily feed intake of RSV group was significantly higher than that of EW and CON groups ($P < 0.05$). In terms of ADG and feed-to-weight ratio, the RSV group was higher than the EW and CON groups, but the differences among the three groups were not significant ($P > 0.05$). From 1 to 60 days of age, the EW and RSV groups showed a decreasing trend from 31 to 45 days of age, whereas the ADG of lambs in the CON group increased gradually with age, and then showed a decreasing trend after 61 days of age. The ADG of lakesheep lambs in the EW and RSV groups declined to varying degrees after weaning, whereas that of lambs in the CON group showed an increase in the ADG after weaning.

Table 5. Effects of different weaning ages on growth performance in Hu sheep lambs.

Items	Days of age	CON group	EW group	RSV group	*P value*
Birth weight/kg	1	3.55 ± 0.17	3.57 ± 0.17	3.58 ± 0.17	0.181
Last weight/kg	90	19.88 ± 0.98a	21.10 ± 1.02b	20.05 ± 0.10b	0.049
ADG/g	1~15	118.21 ± 8.54	120.90 ± 10.83	127.64 ± 8.75	0.133
	16~30	132.53 ± 11.77	163.22 ± 14.28	160.40 ± 12.77	0.204
	31~45	195.83 ± 16.90a	155.84 ± 17.16b	153.99 ± 23.42b	0.012
	46~60	228.40 ± 19.47	217.44 ± 111.73	235.51 ± 20.45	0.199
	61~75	129.38 ± 12.47	138.97 ± 19.26	150.15 ± 15.73	0.311
	76~90	197.41 ± 17.45	218.82 ± 26.57	233.54 ± 18.48	0.220
	1~90	166.96 ± 18.72	169.20 ± 16.58	170.21 ± 20.83	
ADFI/g	1~90	871.04 ± 66.07b	891.04 ± 60.59b	936.36 ± 50.06a	0.014
F/G	1~90	5.22 ± 0.48	5.30 ± 0.36	5.16 ± 0.40	0.023

Note: Different lowercase letters indicate significant differences among treatments ($P < 0.05$), while with the same or no letter superscripts mean no significant difference ($P > 0.05$). The same as below.

4.2. Effects of early weaning and nutritional interventions on digestion and metabolism in lambs

As shown in **Table 6**, there was no significant difference in the CP apparent digestibility of milk replacer on DM, NDF, and ADF in the 3 groups of lambs ($P > 0.05$), and the apparent digestibility of CP in the EW group was significantly lower than that of the RSV and CON groups ($P < 0.05$). In contrast, the apparent digestibility of DM in the CON group was higher than that of the EW and RSV groups, while the apparent digestibility of ADF was lower than that of the 2 experimental groups. The highest apparent digestibility of NDF in the RSV group was 72.43%, which was 3.44% and 2.43% higher than that of the EW group and the CON group, respectively.

As shown in **Table 7**, under the same management conditions, the nitrogen intake, deposited nitrogen, net protein utilization and protein biological value indexes of Hu sheep lambs in the experimental groups differed significantly ($P < 0.05$) compared with the control group. The nitrogen intake of the two experimental groups was significantly higher than that of the control group ($P < 0.05$), while the net protein utilization and protein biological value were significantly lower than that of the control group ($P < 0.05$), and the content of deposited nitrogen of the EW group was significantly higher than that of the RSV group and the CON group ($P < 0.05$), with an increase of 24.83% and 18.10%, respectively. Other nitrogen metabolism indexes of lambs in the control and experimental groups did not differ significantly ($P > 0.05$).

Table 6. Effects of different weaning ages on apparent digestibility of nutrients in Hu sheep lambs (DM basis) %.

Items	CON group	EW group	RSV group	*P value*
DM	72.59 ± 0.43	71.91 ± 0.28	71.25 ± 1.34	0.023
NDF	70.71 ± 1.18	70.02 ± 1.44	72.43 ± 1.08	0.329
ADF	38.15 ± 1.93	40.77 ± 3.43	38.63 ± 2.87	0.145
CP	57.50 ± 1.58b	51.87 ± 0.94a	55.37 ± 0.56b	0.011

Table 7. Effects of different weaning ages on nitrogen metabolism in Hu sheep (DM basis) %.

Items	CON group	EW group	RSV group	*P value*
Nitrogen intake/(g/d)	29.39 ± 0.52b	34.60 ± 0.89a	44.53 ± 0.34c	0.023
Fecal nitrogen/(g/d)	12.34 ± 0.62	16.30 ± 0.99	21.20 ± 0.31	0.096
Urine nitrogen/(g/d)	4.45 ± 0.84	6.38 ± 0.91	8.45 ± 0.05	0.125
Total discharged nitrogen/(g/d)	16.79 ± 1.09	22.68 ± 0.58	29.65 ± 1.44	0.079
Nitrogen retention/(g/d)	12.60 ± 0.90b	11.92 ± 1.03a	14.88 ± 0.27b	0.013
NPU/%	58.01 ± 2.22a	52.89 ± 1.67b	52.39 ± 2.28b	0.018
BV/%	42.87 ± 0.65a	34.45 ± 0.54b	33.42 ± 0.61b	0.032

4.3. Effect of early weaning and nutritional intervention on serum indices in lambs

As shown in **Table 8**, with the prolongation of weaning age and nutritional interventions, the serum GLU, HDL-C, LDL-C, and TC contents of Hu sheep lambs gradually decreased on the day of weaning. At 90 days of age, the serum GLU content of the RSV group was significantly higher than that of the CON group ($P < 0.05$). On the day of weaning, the serum HDL-C content was the highest in the EW group, which was highly significantly higher than that in the RSV and CON groups ($P < 0.01$), and significantly higher in the RSV group than that in the CON group ($P < 0.05$). On the day of weaning, the serum LDL-C content of the EW group was significantly higher than that of the CON group ($P < 0.01$), and significantly higher than that of the RSV group ($P < 0.05$). On the day of weaning, the serum TC content was significantly higher in the EW group than in the RSV and CON groups ($P < 0.05$), and the serum TG content was highly significant in the RSV group than in the EW and CON groups ($P < 0.01$).

Meanwhile, on the day of weaning, the RSV group had the highest serum ALP activity and the lowest ALT activity, and the serum ALP activity of the RSV group was significantly higher than that of the CON group ($P < 0.05$), and there was no significant difference in the ALP activity in the other time periods ($P > 0.05$), and there was no significant effect of the different weaning ages and nutritional interventions on the serum ALT activity of Hu sheep lambs ($P > 0.05$).

Table 8. Effects of different weaning ages on serum biochemical indexes in Hu sheep lambs.

Items	Time	CON group	EW group	RSV group	*P value*
GLU/(mmol/L)	Day of weaning	3.91 ± 0.47	4.98 ± 0.60	3.92 ± 0.36	0.198
	90 days of age	2.13 ± 0.30b	2.46 ± 0.29ab	3.28 ± 0.41a	0.045
HDLC/(mmol/L)	Day of weaning	0.69 ± 0.13c	1.38 ± 0.17a	1.01 ± 0.09b	0.003
	90 days of age	0.44 ± 0.02	0.44 ± 0.05	0.48 ± 0.07	0.256
LDLC/(mmol/L)	Day of weaning	0.65 ± 0.15b	1.11 ± 0.06a	0.75 ± 0.08ab	0.005
	90 days of age	0.22 ± 0.03	0.65 ± 0.15	0.51 ± 0.11	0.332
TC/(mmol/L)	Day of weaning	1.34 ± 0.29b	2.18 ± 0.34b	1.48 ± 0.17a	0.008
	90 days of age	0.71 ± 0.08	0.75 ± 0.09	0.78 ± 0.14	0.296

Table 8. (*Continued*).

Items	Time	CON group	EW group	RSV group	*P value*
TG/(mmol/L)	Day of weaning	0.53 ± 0.07b	0.71 ± 0.15b	1.02 ± 0.14a	0.003
	90 days of age	0.21 ± 0.03	0.17 ± 0.08	0.22 ± 0.04	0.366
ALP/(mmol/L)	Day of weaning	684.06 ± 100.47b	951.10 ± 414.85ab	1074.64 ± 244.70a	0.042
	90 days of age	344.04 ± 70.79	441.42 ± 73.70	608.15 ± 68.85	0.306
ALT/(U/L)	Day of weaning	7.08 ± 1.97	10.17 ± 1.48	5.77 ± 2.53	0.122
	90 days of age	6.78 ± 2.00	7.19 ± 2.56	8.82 ± 6.17	0.231
TP/(g/L)	Day of weaning	47.94 ± 7.01	49.14 ± 5.93	54.98 ± 4.16	0.246
	90 days of age	42.89 ± 4.98	33.56 ± 4.22	39.34 ± 5.92	0.223
ALB/(g/L)	Day of weaning	24.98 ± 1.89	28.36 ± 2.96	26.52 ± 1.37	0.118
	90 days of age	20.03 ± 1.17	19.93 ± 1.35	21.37 ± 2.04	1.243
GLB/(g/L)	Day of weaning	25.71 ± 5.93	20.78 ± 3.68	28.46 ± 3.79	0.332
	90 days of age	22.86 ± 4.53	13.32 ± 3.68	17.97 ± 4.47	0.219

5. Discussion

The key to successful weaning of lambs is to minimize weaning stress and ensure healthy growth after weaning, different weaning ages will affect the growth and development of lambs. Early weaning will make the lambs weak and susceptible to various diseases, which may even lead to death in serious cases; late weaning will lead to delayed recovery of the ewes and a decline in production performance.

Daily weight gain is one of the best indicators of growth and acclimatization of sheep under early weaning conditions [18]. It was found that at 16–30 days of age, EW and RSV groups were weaned at the same time and RSV group was given nutritional intervention, the mean daily weight gain of lambs in EW group was not significantly different from RSV group at this stage. At 31–45 days of age, the ADG of lambs in the EW group was significantly lower than that of the CON group, and the ADG of lambs in both the EW and RSV groups decreased to different degrees after weaning, with a decrease of 5.16% in the EW group and a decrease of 21.30% in the RSV group. In contrast, the ADG of the CON group increased by 16.63% after weaning. This indicated that early weaned lambs were more susceptible to weaning stress than late weaned lambs.

In this experiment, the EW and RSV groups showed a strong growth advantage in the late stage of the experiment, and it could be seen that compared with the lambs weaned at a late stage, the lambs weaned at an early stage had a higher ADG in the late stage of the growth period, which might be due to the fact that the experimental group weaned at an early stage, and the adaptability to the external environment was gradually enhanced, from the period of fattening to the end of the experiment, the ADG of the EW group and the RSV group was higher than that of the CON group in the period from 61 d to 90 d. The ADG of the EW group was higher than that of the CON group in the period from the fattening period to the end of the experiment. The ADG of EW and RSV groups was higher than that of CON group from 61 d to 90 d. In addition, the ADG of RSV group was higher than that of EW group from 46 d to 90 d, which indicated that the nutritional intervention had a slight effect on the ADG

of lambs in the late stage of the experiment. However, at 90 days of age, the differences in ADG among the three groups of weaned lambs were not significant, and the ADG of lambs was higher in the RSV group, EW group, and CON group in descending order, with the RSV group and the EW group showing greater growth potential. The feed-to-weight ratio is an important parameter for measuring feed remuneration and feed conversion ratio. In this experiment, the highest feed-to-weight ratio was 5.30 in the EW group, the second highest was 5.22 in the CON group, and the lowest was 5.16 in the RSV group, suggesting that the EW group of lake lambs used more feed and gained less weight, while the RSV group of lake lambs used less feed and gained more weight.

Supplementary feeding of early weaned lambs with milk replacer and milk replacer with high nutritive value and good palatability can increase their feed intake and contribute to the increase of rumen volume and metabolic capacity of weaned lambs [19]. Any damage to the intestinal tract can trigger serious health problems in the organism, and the stress resulting from separation from the mother at early weaning can cause impairments in the functioning of the intestinal tract and immune system of the lambs [20]. Apparent digestibility of nutrients can reflect the gastrointestinal characteristics of lambs and their ability to digest and absorb nutrients. Due to the effect of weaning stress on the development of gastrointestinal tract, in addition to different weaning ages preweaning milk intake of lambs, nutrient composition of milk replacer, and physical form of food intake also affect the nutrient intake, digestion and metabolism of cap lambs. The apparent digestibility of CP reflects the degree of absorption and utilization of protein in feed for the early weaned cap lambs.

In this experiment, the apparent CP digestibility of the control group was significantly higher than that of the two experimental groups, so it can be seen that the prolongation of weaning age led to an increase in the apparent CP digestibility, which may be due to the higher apparent digestibility of NDF and ADF in the two experimental groups, which prevented the contact of digestive enzymes with proteins, and led to a decrease in the digestibility of proteins in the experimental groups; It is also possible that the lambs in the experimental group had higher feed intake, and the digestive enzymes secreted in the body could not meet the amount needed to digest a large amount of proteins, and the imbalance of digestive substrates and enzymes directly affected the decomposition effect of proteins, which in turn affected the digestibility of proteins. In addition, it was found that different weaning ages and nutritional interventions did not significantly affect the apparent digestibility of other major nutrients except CP, which may be due to the fact that early supplementation of solid feeds promotes the development of the digestive tract.

Nitrogen is a nutrient critical for growth and development of ruminants. There is a significant negative correlation between nitrogen intake and nitrogen utilization efficiency in lambs [21]. This is consistent with the results of the present experimental study. Lambs in the three groups showed a high degree of consistency in nitrogen metabolism except for deposited nitrogen. Higher nitrogen intake in lambs resulted in higher levels of nitrogen-like nitrogen, urinary nitrogen, and total excreted nitrogen, and lower net protein utilization and biological value of proteins. The results of the present experimental study were consistent with the results of the present experimental

study. From the experimental results, it can be seen that the RSV group had the highest intake of nitrogen and the lowest biological value of protein, which may be due to the fact that the weaned lambs in the RSV group had a higher growth rate, resulting in a high loss of nitrogen, which lowered the metabolizing efficiency of nitrogen in the weaned lambs. Early weaning can increase the feed intake of lambs and promote the growth and development of lambs, and milk replacer can promote the development of rumen of cap lambs through physical stimulation to improve the intestinal digestive organs. Moreover, the increase in nitrogen intake of Hu sheep lambs is favorable to nitrogen deposition.

TG, TC, GLU, HDL-C, LDL-C are important indicators of lipid metabolism in the body. GLU levels reflect energy utilization in ruminants. When serum GLU level is reduced, it indicates feed energy deficiency or poor energy utilization [22]. Elevated serum cortisol levels when animals are subjected to stress result in increased GLU levels. In the present study, serum GLU levels were lower in all three groups of lambs at 90 days of age compared to the day of weaning and were significantly higher in the test group than in the control group. In addition, it was found that after weaning, the serum TC content of the test group was significantly higher than that of the control group, so it can be inferred that the test group had more body fat deposition and lower lean meat rate than the control group. Moreover, the serum HDL-C, LDL-C and TG contents of lambs in all three groups were lower than those before weaning, indicating that the nutritional requirements of lambs need to be maintained by fat metabolism after weaning due to the change of nutrient acquisition from goat's milk to milk replacer as well as the weaning of lambs.

ALT and ALP mainly reflect the health of the liver. The serum ALT activity of the three groups of lambs showed a decreasing trend at 90 days of age compared with that before weaning. Different weaning days and nutritional interventions had no significant effect on serum ALT activity in lake lambs. This indicates that different weaning days and feeding methods do not affect the liver function of lambs, and with the increase of age, the lambs' body development is perfect, which is favorable to the healthy growth of lambs. Serum ALP can be used as an indicator of bone growth in lambs, and the higher its activity, the more favorable it is to the growth and development of the animal [22]. In this experiment, the serum ALP activity of the test group on the day of weaning was significantly higher than that of the control group, which indicated that early weaning (21 d) could promote the growth of lambs.

6. Conclusion

Stress was caused to lambs after weaning at 21 days of age. Early weaning from breast milk resulted in a significant increase in feed intake but a decrease in daily weight gain, and after acclimatization early weaned lambs showed strong growth later in the trial (65–90). The nutritional intervention (feeding resveratrol) did not significant significantly on the growth performance of the lambs. Apparent CP digestibility increased with longer weaning age, but weaning age did not have a significant effect on the apparent digestibility of other major nutrients, and early resveratrol feeding did not play a significant role in improving nutrient digestion and metabolism. Early weaning and addition of resveratrol had a significant effect on

serum GLU and TC indexes of lambs, but did not show a significant effect on other indexes.

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Ethical approval: The study was conducted in accordance with the Declaration of Helsinki. The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of TOPBIOTECH IACUC (protocol code TOP-IACUC-2023-0221, and date of approval 2023-9-1).

Conflict of interest: The authors declare no conflict of interest.

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