



Original research article

Effects of weaning age on growth, nutrient digestibility and metabolism, and serum parameters in Hu lambs



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ABSTRACT

This study was conducted to investigate the effect of weaning age on growth performance, nutrient digestion and metabolism, and serological indicators, and to obtain an optimal weaning age in Hu lambs. Forty-eight newborn Hu lambs (birth weight, 2.53 ± 0.14 kg) were randomly divided into 4 groups. The lambs in control group (ER) suckled their dams. The lambs in other three experimental groups were weaned on milk replacer at 10, 20, and 30 days of age (EW10, EW20, and EW30 groups), respectively. The results were as follows: 1) lambs in EW10 and EW30 groups had a lower ($P < 0.05$) ADG than those in ER group within 10 days post-weaning; the weaned lambs began to show a higher ($P < 0.05$) ADG than those in ER group after 20 days post-weaning. 2) EW10 and EW20 groups had a higher ($P < 0.05$) creep feed intake than EW30 and ER groups from 15 to 60 days of age. 3) The apparent digestibility of dry matter, organic matter, gross energy, nitrogen, ether extract and phosphorus, and the deposition of nitrogen and phosphorus did not differ ($P > 0.05$) among groups; however, the apparent digestibility and deposition of calcium in early weaned lambs were lower ($P < 0.05$) than those in ewe-reared lambs. 4) The albumin content in EW30 group was lower ($P < 0.05$) than that in ER group; the globulin content in EW30 group was higher ($P < 0.05$) than that in other groups; the content of serum insulin-like growth factor-I in weaned lambs tended to increase compared with lambs in ER group. Finally, the growth rate of lambs decreased within 10 days post-weaning, but early weaning boosted creep feed intake, leading to better growth and health later in life. The Hu lambs can be weaned on milk replacer and creep feed at 10 days of age.

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1. Introduction

Early-weaning of lambs has become a key process for improving the efficiency and profitability of the intensive sheep husbandry. Weaning age, as one of the key parameters in early weaning, is crucial for the success of early-weaning of lambs (Teke and Akdag,

2012). Due to underdevelopment and rapidly growth of gastrointestinal tract, it is essential to obtain the best weaning age to minimize weaning stress and guarantee post-weaning healthy growth of lambs. Weaning lambs on milk replacer (MR) instead of solid feed has been steadily gaining ground. However, results on weaning age of lambs vary widely because of the diversity of feeding and management practices and the various genotypes of lambs. Lanza et al. (2006) weaned lambs at 1 day of age and fed them a MR. Yeom et al. (2002) carried out early weaning at 7 days of age. Most previous studies examined the growth rate for a single period, instead of focussing on the growth curve and nutrient metabolism of weaned lambs throughout their production cycle. In addition, weaning at different ages would result in different types of weaning stress, which would affect subsequent growth and health of lambs. It is necessary to find the optimal weaning age for the specific lamb under a specific feeding and management

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condition. Hu sheep are a prolific indigenous mutton breed in China. However, the weaning age of Hu lambs has not been fully examined (Chen et al., 2012). Therefore, we hypothesized that weaning the Hu lamb on MR at an appropriate age could minimize weaning stress and benefit their later growth. Thus, the objective of this study was to investigate the effect of different weaning ages on growth performance, nutrient digestibility, and serum parameters in Hu lambs, and to obtain an optimal weaning age for improving the efficiency and profitability of intensive Hu lamb production.

2. Materials and methods

The experimental procedure was approved by the Animal Ethics Committee of CAAS, and humane animal care and handling procedures were followed throughout the experiment. This study was conducted on the farm of Taizhou Helen Sheep Ltd. in Jiangsu Province, China (latitude 32°44'N, longitude 120°24'E), from October to December in 2013.

2.1. Experimental design, animal and diets

A single factor randomized design was applied and weaning age of lambs was taken as the experimental factor. Forty-eight Hu lambs (birth weight, 2.53 ± 0.13 kg; 24 males and 24 females) were selected based on their birth type, gender, and birth weight and randomly assigned into 4 groups. Each group had 3 replicates with 4 lambs per replicate. The lambs were ewe-reared (ER group), or early weaned at 10, 20 and 30 days of age (EW10, EW20, and EW30 groups) and fed a MR (Beijing Precision Animal Nutrition Research Centre, patent number ZL02 128844.5) until 60 days of age. All lambs had ad libitum access to a commercial textured creep feed from 15 to 60 days of age, and their creep feed intakes were recorded daily. The nutrient levels of ewe milk, MR, and creep feed are presented in Table 1. Each lamb was weighed at 10, 20, 30, 40, 50 and 60 days of age and average daily gains (ADG) were calculated.

2.2. Raising management

Ewe-reared lambs stayed with their dams during the whole experimental period but were not allowed to access the ewes' diet. Other weaned lambs were separated from their dams with 4 lambs in a 4×2 m² indoor pen. A 3-day adaptation was carried out to wean lambs gradually from their dams. During the adaptation period, MR was offered gradually according to the body weight of lambs (0.67% of BW for d 1, 1.34% of BW for d 2, and 2% of BW for d 3) before the ewe's milk was totally replaced. The amount of MR given after weaning was adjusted according to the lamb BW

(approximately 2%) every 10 days with 3 times daily (0700, 1300, and 1900 h) from 10 to 50 days of age and then 2 times daily (0800, 1800 h) from 51 to 60 days of age. Before feeding, the MR was dissolved in hot water to obtain a 40 °C solution (16.67% dry matter) and offered to each animal with a milk bottle. Clean water was offered ad libitum throughout the trial.

2.3. Sampling and analyses

A digestibility and metabolism trial was carried out from 50 to 60 days of age with 4 lambs randomly selected from each group. Lambs were moved from the floor pens into individual metabolism crates at 50 days of age. During the digestibility trial period, the ewe-reared lambs were put back to their dams to ingest ewe milk about 10 min whereas the weaned lambs feeding with MR. After 5 days of adaptation, the amounts of feed intake, faeces and urine were recorded from 56 to 60 days of age. During this time, total faeces were collected and weighed daily. After thorough and uniform mixing, 10% of total faeces was taken and mixed with a few millilitres of 12 mol/L HCL solution. The faecal samples from each lamb were pooled over the 5-day collection period. Total urine was collected into a plastic container containing 100 mL of 12 mol/L HCL solution to maintain pH below 3, the volume was measured, and recorded daily. Then 20% of the total urine was collected and pooled after the 5-day collection period. Samples of feed orts, faeces and urine were frozen in the refrigerator at -20°C for further analysis. After the digestibility trial, the frozen feed, orts, and faecal subsamples were thawed overnight at room temperature and analysed for air dry matter by drying in an oven at 65°C for 48 h and ground through a 1-mm sieve. The samples were then analysed for dry matter by drying in an oven at 135°C for 2 h, organic matter by ashing at 550°C for at least 8 h, crude protein using the macro-Kjeldahl procedure that was calculated as $6.25 \times \text{N}$, ether extract using a reflux system (ANKOM XT15, America) with petroleum ether at 90°C for 1 h, gross energy through an isothermic calorimeter (6400 Calorimeter, Parr Instrument Company, Illinois, USA), calcium using an atomic absorption spectrometer (Czerny-Turner AAS8000, America), and phosphorus determined by the molybdenum blue colorimetric method (AOAC, 1999).

At 60 days of age, blood samples obtained from 4 lambs per group were collected in heparinized tubes by jugular venipuncture and centrifuged within 2 h at $1,040 \times g$ for 15 min to obtain serum (which was then stored at -20°C for further analysis). The serum concentrations of total protein, albumin, globulin, serum urea nitrogen (SUN), cholesterol, glucose, non-esterified fatty acid (NEFA), triglyceride and alkaline phosphatase (ALP) activity were determined using an automated analyzer (Hitachi 7600, Tokyo, Japan). Insulin-like growth factor I (IGF-I) concentration was determined using enzyme-linked immunosorbent assay (ELISA) kits (R&D System Inc., Minneapolis, MN, USA).

2.4. Statistical analysis

The data of ADG and creep feed intake were analysed using the PROC MIXED procedure of one-way ANOVA with repeated measures of SAS software and conducted double comparative using LSD method (Version 9.2, SAS Institute Inc., Cary, NC) according to the following model: $Y_{ijk} = \mu + T_i + M_j + TM_{ij} + C(T)_{ik} + \varepsilon_{ij}$, where Y was the dependent variable, μ was the overall mean, T was the effect of group, M was the age after birth, C was the effect of lambs, and ε was the residual error. Group differences were considered significant when $P < 0.05$ and tendencies were discussed when $0.05 < P < 0.10$.

Data for nutrient utilization and serum parameters were analysed as a completely randomized design using one-way analysis of

Table 1
Nutrient levels of ewe milk, milk replacer and creep feed (DM basis).¹

Item	Ewe milk	Milk replacer	Creep feed
DM, %	31.12	94.47	86.37
ME, MJ/kg	19.11	17.69	13.30
CP, %	24.71	24.80	19.58
EE, %	45.27	15.43	3.77
Ash, %	4.14	7.70	8.52
Ca, %	3.70	1.02	0.95
TP, %	0.72	0.66	0.70

DM = dry matter; ME = metabolic energy; CP = crude protein; EE = ether extract; TP = total phosphorus.

¹ Nutrient levels were measured except ME. Metabolic energy of milk replacer was calculated according to the results of Wang et al. (2015) and NRC (2007), and that of creep feed was calculated according to Table of Feed Composition and Nutritive Values in China 2012 and NRC (2007), and that of ewe milk was calculated according to the results of digestive and metabolic experiments in the current study.

variance (Version 9.2, SAS Institute Inc., Cary, NC). Duncan's method for multiple comparisons was used if the overall F test for the measurement was significant ($P < 0.05$). The results of all data were stated as least-squares means.

3. Results

3.1. Effect of weaning age on growth performance

3.1.1. Lamb ADG

The effect of weaning age on the ADG of lambs is showed in Table 2. The ADG in EW10 group decreased ($P < 0.05$) from 11 to 20 days of age, whereas it increased ($P < 0.05$) from 31 to 40 days of age compared with other groups. The ADG in EW30 group was lower ($P < 0.05$) from 31 to 40 days of age, whereas it was greater from 51 to 60 days of age than that in EW10 and ER groups. The ADG in EW20 group was greater from 41 to 50 and 51 to 60 days of age than that in ER group.

The ADG of lambs among 4 groups was not different ($P > 0.05$) during the whole trial period and from 1 to 10 and 21 to 30 days of age. However, it significantly increased with the increasing age ($P < 0.01$), and there was a significant interaction effect between group and age.

3.1.2. Lamb creep feed intake

The effect of weaning age on creep feed intake is reported in Table 3. Weaning age had a significant effect on feed intake and there was an interaction effect between group and age. The creep feed intake in EW10 group was not different from ER group from 15 to 20 days of age. However, it increased ($P < 0.05$) from 21 to 60 days of age compared with ER group. The creep feed intake of EW20 group was greater ($P < 0.05$) than that of ER group from 31 to 60 days of age. During the whole trial period, the creep feed intake of EW10 and EW20 groups was greater ($P < 0.05$) than those of ER group. Furthermore, creep feed intake in EW10 group was greater ($P < 0.05$) than that in EW20 group. However, there was no difference ($P > 0.05$) between EW30 and ER groups.

3.2. Effect of weaning age on nutrient digestibility and metabolism

The apparent digestibility of dry matter, organic matter, gross energy, nitrogen, ether extract and phosphorus, and the deposition of nitrogen and phosphorus was not different ($P > 0.05$) among groups (Table 4). The weaned lambs had a lower ($P < 0.05$) apparent digestibility and deposition of calcium compared with ewe-reared lambs.

3.3. Effect of weaning age on serum parameters

Effect of weaning age on serum parameters of lambs is presented in Table 5. The ALB concentration was lower ($P < 0.05$) in

EW30 group than that in ER group. The GLB concentration in the EW30 group was higher ($P < 0.05$) than that in other groups. The GLB in EW10 and EW20 groups was greater ($P < 0.05$) than that in ER group. No difference in the serum concentrations of GLU, TP, SUN, cholesterol, triglyceride, NEFA, IGF-I and ALP was observed among groups. However, the concentration of IGF-I in weaned lambs was trended to increase ($P < 0.10$) compared with ewe-reared lambs.

4. Discussion

4.1. Effect of weaning age on growth performance in lambs

In the current study, the growth rate of weaned lambs decreased at first 10-day post-weaning compared with ewe-reared lambs. It was the weaning stress caused by separating from their dams, changing of diets and feeding regime that limited the lamb growth (Napolitano et al., 2008). Emsen et al. (2004) reported that lambs weaned at 2 or 3 days of age had a lower BW at 15 days of age than ewe-reared lambs. After 10 days of post-weaning, it appeared that the early-weaned lambs were used to the new feeding regime and rearing environment in our study. Meanwhile, the nutrient content of MR maybe fulfilled the nutrient requirement of lambs in the later period. All these led to a greater ADG in early-weaned lambs. Similarly, Bhatt et al. (2009) founded that early weaned lambs had a higher BW in later period of post-weaning. Emsen et al. (2004) also reported that the group fed MR had a more rapid growth than the control after lambs adapting new feeding regime. Furthermore, lambs weaned at 10 days of age had a faster recoverability to grow. It was speculated that the earlier weaning of lambs resulted in a smaller psychological stress from the separation with the ewes (Simitzis et al., 2012) and an ability to consume more creep feed (Álvarez-Rodríguez et al., 2010).

Weaning on ewe milk and feeding MR can boost creep feed intake. In this study, lambs in EW10 and EW20 groups had a greater creep feed intake than lambs in EW30 and ER groups, especially the EW10 lambs. The creep feed intake was correlated with rumen development. The rumen function establishment of lambs begins at 3 weeks of age when rumen epithelial cell proliferation is sensitive to the stimulus of plant protein or solid feed. Ghorbani et al. (2007) reported that feeding MR was important for the development of rumen of lambs and therefore improved creep intake ensued. Similarly, Álvarez-Rodríguez et al. (2010) also confirmed that early-weaned lambs had a higher creep feed intake than suckling lambs. Therefore, earlier weaning on MR and subsequent higher creep feed intake in EW10 and EW20 groups should stimulate and enhance the earlier rumen development, which leads to a greater growth rate. However, the higher creep feed intake was not observed in lambs weaned at 30 days of age. It is speculated that the long time lambs relying on

Table 2
Effect of weaning age on the average daily gain (g/d) of lambs.

Age, d	Group ¹				SEM	P-value		
	EW10	EW20	EW30	ER		Age	Group	Age × group
1 to 60	190.49	180.27	178.75	171.26	5.36	<0.0001	0.3794	<0.0001
1 to 10	190.17	195.73	207.00	199.09	10.38			
11 to 20	56.25 ^a	129.73 ^b	116.42 ^b	107.00 ^b	9.48			
21 to 30	104.42	82.64	94.33	90.45	8.92			
31 to 40	271.75 ^a	115.73 ^{bc}	76.83 ^c	193.55 ^b	11.50			
41 to 50	252.50 ^a	264.91 ^a	238.33 ^{ab}	204.27 ^b	11.41			
51 to 60	270.83 ^b	305.82 ^{ab}	335.58 ^a	240.55 ^c	10.75			

^{a,b,c} Within a row, means without a common superscript differ significantly ($P < 0.05$).

¹ The lambs were ewe-reared (ER group), or early weaned at 10, 20 and 30 days of age (EW10, EW20, and EW30 groups) and fed with milk replacer.

Table 3
Effect of weaning age on creep feed intake [g/(d·lamb)] of lambs.

Age, d	Group ¹				SEM	P-value		
	EW10	EW20	EW30	ER		Age	Group	Age × group
15 to 60	260.33 ^a	182.91 ^b	154.75 ^c	153.75 ^c	8.13	<0.0001	<0.0001	<0.0001
15 to 20	32.38	10.97	10.18	11.28	1.46			
21 to 30	126.69 ^a	41.18 ^b	30.17 ^b	29.07 ^b	6.43			
31 to 40	251.37 ^a	184.61 ^b	116.26 ^c	91.47 ^c	9.56			
41 to 50	367.34 ^a	289.08 ^b	268.55 ^c	250.75 ^c	8.96			
51 to 60	523.87 ^a	388.71 ^b	398.58 ^b	386.19 ^b	10.81			

^{a,b,c} Within a row, means without a common superscript differ significantly ($P < 0.05$).

¹ The lambs were ewe-reared (ER group), or early weaned at 10, 20 and 30 days of age (EW10, EW20, and EW30 groups) and fed with milk replacer.

their dams may have retarded their adaption to the MR and creep feed.

4.2. Effect of weaning age on nutrient utilization in lambs

Nutrient utilization can reflect the lamb gastrointestinal characteristics and productivity. Nutrient digestibility and metabolism of lambs weaned at different ages varies as gastrointestinal development is affected due to weaning stress (Zhong et al., 2014). In the current study, no difference was observed among different treatments on the digestibility and utilization of main nutrients. The ewe-reared lambs reduced their milk intake as time went by and also had a lower intake of creep feed compared with early-weaned lambs. However, the ewe-reared lambs had a lower ADG compared with early-weaned lambs from 50 to 60 days of age. While the early-weaned lambs with high creep feed intake and ADG were observed, the nutrient utilization was likely similar. In a similar study, Danso et al. (2014) reported that feeding MR plus creep significantly increased growth performance and organ development of lambs, however, did not affect the efficiency of feed utilization. The current results were similar to previous studies, suggesting it may be possible for artificially-reared or ewe-reared lambs to have a similar gastrointestinal development. However, the early-weaned lambs had a lower apparent digestibility and deposition of Ca compared with ewe-reared lambs in this study. It was likely due to the inorganic calcium salt added in MR, which affected the Ca digestibility.

4.3. Effect of weaning age on serum parameters in lambs

The serum glucose concentration reflects energy utilisation of ruminants. When serum glucose concentration decreases, it

Table 4
Effect of weaning age on the digestion and deposition of nutrients of lambs (%).

Item	Group ¹				SEM	P-value
	EW10	EW20	EW30	ER		
DM	81.78	83.89	81.82	81.60	0.62	0.5528
OM	83.46	85.44	83.47	84.04	0.74	0.7907
GE	80.96	83.79	82.24	82.74	0.67	0.5630
N	81.46	83.77	81.76	82.92	0.52	0.3924
EE	88.03	91.35	91.15	94.33	1.07	0.2357
Ca	60.29 ^a	52.07 ^a	56.80 ^a	93.52 ^b	4.89	0.0003
CP	77.27	82.61	79.74	83.83	1.85	0.6377
N deposition	52.82	52.98	52.00	53.26	3.85	0.9996
Ca deposition	60.14 ^a	51.76 ^a	56.41 ^a	93.34 ^b	4.91	0.0003
P deposition	65.24	68.21	61.91	74.84	2.36	0.2651

DM = dry matter; OM = organic matter; GE = gross energy; CP = crude protein; EE = ether extract.

^{a,b} Within a row, means without a common superscript differ significantly ($P < 0.05$).

¹ The lambs were ewe-reared (ER group), or early weaned at 10, 20 or 30 days of age (EW10, EW20, and EW30 groups) and fed with milk replacer.

indicates the lack of dietary energy or poor energy utilization. In this study, the serum glucose concentration was not affected which was consistent with the energy digestibility result.

The serum total protein, mainly including albumin and globulin, is the main solid component in serum and is an indicator of the nutritional status of the animal. In this study, TP concentration was not affected by weaning age, which was in accordance with the results of Paez Lama et al. (2014) who reared Criollo kids under two different rearing systems. The serum globulin concentration in early-weaned lambs was higher than that in ewe-reared lambs. Globulin can inactivate and degrade antigen proteins in the body and its content can reflect the animal's immune status (Li et al., 2008). Passive immunity of lambs is inherited from their dams by ingesting colostrum, whereas acquired immunity is developed with the growth and development of lambs (Loste et al., 2008). Therefore, the early weaning of lambs probably strengthened the acquired immunity compared with the ewe-reared lambs and had a high globulin value. However, the immunity of weaned lambs is the topic of future research.

The SUN can reflect the state of protein metabolism in lambs and its concentration is related to dietary nitrogen substance and protein utilization (Stanley et al., 2002). The SUN concentration was not different among the 4 experimental groups. It demonstrated that the N utilization was not affected by the weaning age.

The serum lipid concentration is often correlated with the body lipid metabolism. In the study, serum cholesterol, triglyceride and NEFA concentrations were no difference among groups, which explained the undifferentiated EE digestibility.

As well known, the growth factor, IGF-I, is important for lamb development. In this study, the serum concentration of IGF-I in

Table 5
Effect of weaning age on the serum parameters of lambs.

Item	Group ¹				SEM	P-value
	EW10	EW20	EW30	ER		
Glucose, mmol/L	8.66	7.75	8.70	8.20	0.21	0.2334
Total protein, g/L	49.63	48.23	49.78	43.33	0.88	0.2300
Albumin, g/L	33.94 ^{ab}	31.91 ^{ab}	29.95 ^b	37.55 ^a	0.84	0.0004
Globulin, g/L	15.70 ^b	16.33 ^b	19.83 ^a	7.84 ^c	1.22	0.0028
SUN, mmol/L	11.00	13.24	12.39	12.38	0.84	0.2383
Cholesterol, mmol/L	2.49	2.58	2.12	2.59	0.09	0.3993
Triglyceride, mmol/L	0.36	0.43	0.37	0.35	0.02	0.4710
NEFA, μmol/L	506.76	519.03	556.87	586.71	16.97	0.1668
IGF-I, ng/mL	150.20	121.21	128.65	110.75	7.28	0.0773
ALP, U/L	183.06	172.72	151.87	149.99	13.91	0.2291

SUN = serum urea nitrogen; NEFA = non-esterified fatty acid; IGF-I = Insulin-like growth factor I; ALP = alkaline phosphatase.

^{a,b,c} Within a row, means without a common superscript differ significantly ($P < 0.05$).

¹ The lambs were ewe-reared (ER group), or early weaned at 10, 20 or 30 days of age (EW10, EW20, and EW30 groups) and fed with milk replacer.

weaned lambs trended to increase. Hoffman et al. (2014) stated that animals with poor nutrition will be reduced growth rate because of reduced circulating IGF-I. Therefore, this result was a proof of the great growth rate of weaned lambs.

The serum ALP could be used as a marker of bone growth and is positive correlated with ADG (Hill et al., 2010). Although there was no significant difference among groups, the higher ALP value was apparently correlated with the higher ADG in weaned lambs compared with ewe-reared lambs.

5. Conclusion

In this study, there was a growth check in early-weaned lambs within 10 days post-weaning. After adapting the feeding regime, the weaned lambs fed MR had a higher creep feed intake and growth rate. Overall, the lamb can be weaned on MR at 10 days of age.

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