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Effect of Pre and Post Weaning Diet Quality on Puberty Age and Tail Measures in Kurdish Female Lambs

Sedigheh Menatian, Hamidreza Mirzaei Alamouti, Farshid Fatahnia and Reza Masoumi

Abstract

To determine the value of pre and post weaning nutrition on puberty age, some hormonal concentrations and tail measures in ewe lambs, a total of 40 clinically health Kurdish female lambs (30 ± 8.6 d and weighing 10.2 ± 3.4 kg) were randomly allocated to one of two experimental diets in pre-weaning period: high quality diet (HQD, 2.50 Mcal ME/kg dry matter (DM) and 148 g CP/kg DM) or low quality diet (LQD, 2.02 Mcal ME/kg DM and 87 g CP/kg DM). At weaning, one half of lambs from each group was randomly separated and assigned to HQD or LQD. So there were four treatment groups in post-weaning period: H-H (HQD pre- and post-weaning); H-L (HQD pre-weaning and LQD post-weaning); L-H (LQD pre-weaning and HQD post-weaning) and L-L (LQD pre and post-weaning, control group). Within the post-weaning, serum progesterone concentrations was greater for ewe lambs fed at H-H group than for other groups ($P < 0.05$). Serum insulin concentration was affected by the diet quality at both periods ($P < 0.05$). Leptin concentration was affected by treatment and ewe lambs of L-H group had higher leptin concentrations ($P < 0.05$). Diet plan in the pre-pubertal period was affected tail measures in 120 and 210 days of ages ($P < 0.05$).

Keywords: hormone, Kurdish lambs, milk, nutrition plan, reproductive performance

1. Introduction

In tropical, semiarid, and arid areas, animal production is dependent on supplemental feeding, especially in the reproductive seasons due to the higher energy demand [1]. Reproduction efficiency can play a critical role in determining profit potential for livestock production systems. Most sheep breeds become sexually active in response to decreasing day length in the late summer to early autumn, which is an additional constraint to the timing of puberty in ewe lambs [2]. If a ewe lamb fails to achieve puberty in its first autumn, it will be delayed until the following breeding [3]. Breeding ewes to lamb at 1 year of age is a potential means of improving farm profitability and ewe lifetime performance by reducing the time

interval from birth to first lambing, subsequently reducing feed, labor, housing, and other costs associated with raising replacement animals [4, 5].

Sexual development is an important factor and can be manipulated by altering growth rates [6–8]. During the productive life of ewes, puberty period is critical for both animal health and performance. The onset of puberty in sheep is influenced by genetic and environmental factors such as nutrition, day length, temperature, and their interaction [9]. Ewe lambs growing at faster rates will exhibit their first estrus and are more likely to conceive at a lower age and have heavier body weight (BW) than ewe lambs growing at slower rates [10]. Because of the importance of BW, environmental factors that can affect the rate of growth before and after weaning are important determinants of age at puberty. Mulvaney et al. [11] reported that ewe lambs gaining 208 g/day compared to 153 g/day were more likely to return to breeding, although overall pregnancy rates did not differ. Generally, faster growth is associated with enhanced reproductive performance in ewe lambs, i.e., earlier attainment of puberty, more intense estrous activity, and higher conception and lambing rates when mated [12].

The most economically important traits in sheep production are growth, reproductive performance, and milk production, and there is no study on the abovementioned characteristics in Kurdish ewe lambs; therefore, the objective of this study was to compare the effects of diet quality fed during the pre-weaning and post-weaning periods and potential interactions between pre- and post-weaning diets on skeletal growth, reproduction performance, hormone concentrations, and milk production during the first lactation in Kurdish ewe lambs.

2. Materials and methods

2.1 Hormonal drugs

Controlled internal drug release (CIDR) with 300 mg of progesterone, a pro-gestagen analogue (InterAg, Hamilton, New Zealand), PMSG (Folligon; Intervet International BV, Boxmeer, the Netherlands), oxytocin (Oxytocin V, 10 IU/ml, Phoenix Pharm, Auckland, New Zealand), and commercially available kits of leptin (LDN, Germany, LOT: 150873), insulin (DiaMetra, Italy, LOT N: 3949C), and progesterone (DiaMetra, Italy, LOT N: 4026) were used.

2.2 Locations, animals, and treatment schedule

This study was performed at the Nomadic Management Department, Ilam Province, Iran (33°51' N, 46°27' E), from January 2013 to December 2015. All procedures involving animal care and management were approved by the University of Zanjan Animal Care Committee (proposal no. 1169739). A total of 40 clinically healthy Kurdish female lambs (30 ± 8.6 days and weighing 10.2 ± 3.4 kg) were used. At 30 days of age, lambs were randomly housed together with twice daily access to their mother's milk and were allocated to one of the two experimental treatments to achieve either high or low rates of BW gain during two consecutive periods, from 30 to 120 (pre-weaning period) and from 121 to 210 days of age (post-weaning period). They were kept in individual pens (1 × 2 m) for three consecutive days every 2 weeks for recording dry matter intake (DMI). In pre-weaning period, the lambs were fed high-quality diet (HQD, n = 20) or low-quality diet (LQD, n = 20), and at the weaning time, HQD and LQD fed lambs were re-randomized so that one half of lambs from each group is randomly allocated to HQD or LQD. So there were four treatment groups (n = 10) in post-weaning period: HQD pre- and post-weaning (H-H); HQD pre-weaning and LQD post-weaning (H-L); LQD pre-weaning and

Composition (%)	Pre- and post-weaning diets	
	HQD	LQD
Alfalfa hay	445.1	—
Wheat straw	—	513.7
Ground barley	445.1	428.1
Soybean meal	59.3	—
Calcium carbonate	5.9	6.8
Salt	5.0	5.0
Mineral and vitamin premix*	39.6	46.4
DM	916.0	919.0
CP	148.0	87.0
EE	58.0	22.0
NDF	285.0	450.0
NFC	466.0	371.0
ME (Mcal/kg)	2.50	2.02

Pre- and post-weaning treatments: HQD, high-quality diet; LQD, low-quality diet.
**Each kg (DM basis) of mineral and vitamin premix contained 180 g of Ca, 70 g of P, 35 g of K, 50 g of Na, 58 g of Cl, 30 g of Mg, 32 g of S, 5 g of Mn, 4 g of Fe, 3 g of Zn, 300 mg of Cu, 100 mg of I, 100 mg of Co, 20 mg of Se, 400,000 IU vitamin A, 100,000 IU vitamin D3, and 245 IU vitamin E. DM, dry matter; CP, crude protein; EE, ether extract; NFC, nonfiber carbohydrates = 100 – (CP + NDF + EE + ash); ME, metabolite energy.*

Table 1.
Ingredients and chemical composition of experimental diets.

HQD post-weaning (L-H); and LQD pre- and post-weaning (L-L, control group). The HQD and LQD were formulated according to recommended nutrient requirements for small ruminants [13] that received a diet that covered nutrient requirements including energy and protein needs for a 20 kg growing lamb with an average daily gain (ADG) of 200 and 100 g/day, respectively. Diets were formulated to have different metabolize energy (ME) and crude protein (CP) contents. The HQD and LQD were contained 2.50 and 2.02 Mcal ME /kg DM and 14.9 and 8.9% CP (DM basis), respectively. Rations were totally hand-mixed for each pen and offered in equal proportions twice daily at 09:00 and 16:00 in pre- and post-weaning period. The ingredients and chemical composition of the experimental diets are shown in Table 1.

2.3 Estrous synchronization and pregnancy diagnosis

When ewe lambs reached 210 days old, estrus was induced and synchronized by CIDR. Animals were treated with CIDR for 14 days and were injected with 500 IU PMSG at the time of CIDR withdrawal. Twenty-four hours after CIDR withdrawal, all of ewe lambs were monitored for estrus detection by five intact fertile rams and were ultimately naturally bred. The rams remained with the ewe lambs until the termination of estrous signs. After serving, all ewe lambs were kept together in the same nutritional and managerial conditions and reared in the pasture until 2 weeks before expected parturition. Pregnancy diagnosis was determined by using of transabdominal ultrasound (Pie Medical, Falco 100, Netherlands) at 60 days after serving.

2.4 Data collection and calculation

BW was measured every 2 weeks from 30 to 210 days of age. Feed offered and feed refusals of individual pens were weighed and recorded daily, and DM content

of total mixed ration (TMR) and orts was determined to estimate DMI. ME and CP intake were calculated as DMI from each diet multiplied by their ME and CP contents, respectively [13]. DM, CP, and ether extract (EE) of experimental diets were measured according to the methods of AOAC [14]. The neutral detergent fiber (NDF) was measured according to the method described by Van Soest et al. [15] without α -amylase and sodium sulfite and was expressed exclusive of residual ash. Nonfibrous carbohydrates (NFC) were calculated according to NRC [16] dairy cattle model as $100 - (\text{CP} + \text{NDF} + \text{EE} + \text{ash})$. Milk intake by ewe lambs was measured by the weigh-suckle-weigh method (WSW) in three consecutive days every 2 weeks from the start of study to weaning (30–120 days). At the start of WSW method at each suckling occasion (twice daily), ewe lambs were weighed, allowed to suckle the udder of their dams, and weighed again immediately after suckling. The difference between pre- and post-suckling weights was defined as milk intake (2 months [17]). On each milking occasion, ewes were milked by hand after intravenous injection of 1 IU synthetic oxytocin. Milk samples of dams in subsequent lactation were collected in three consecutive days every 2 weeks and analyzed for fat, protein, and lactose by using of MilkoScan 133B (Foss Electric, Hillerød, Denmark). Milk protein, fat, and lactose yields were calculated by multiplying milk yield from the respective day by protein, fat, and lactose contents of the milk for each ewe. Milk gross energy (GE) was calculated as $\text{GE} = ((0.0547 \times \text{CP}\%) + (0.0929 \times \text{Fat}\%) + (0.0395 \times \text{Lactose}\%))$ according to NRC [16]. The mean metabolize ability of the ewe milk GE is 0.94 [18]; therefore, milk ME content was calculated as $\text{GE} \times 0.94$. Energy-corrected milk (ECM) and fat-corrected milk (6.5% FCM) were calculated as $\text{ECM} = (0.327 \times \text{kg milk}) + (12.95 \times \text{kg fat}) + (7.2 \times \text{kg protein})$ and $\text{FCM} = \text{milk yield} \times (0.37 + (0.097 \times \text{Fat}\%))$.

2.5 Blood sampling and analysis

Before the first meal of the day, blood samples (5 ml) were collected by jugular venipuncture from each lamb every 2 weeks from 90 days of age until puberty (age at puberty was assessed by serum concentrations of progesterone, where puberty was determined as the age when two consecutive blood samples contained at least 1 ng of progesterone/mL). Hence, samples were centrifuged for 15 min (3000 rpm), and sera were separated into 1.5 ml micro tubes and then placed in freezer (-20°C). Serum samples were tested for leptin, insulin, and progesterone by ELISA method. Standard commercial kits were used for analysis, and the procedures were adopted as recommended by the manufacturer of these kits.

2.6 Statistical analyses

Data were analyzed as a completely randomized design in factorial arrangement (2×2) by using the mixed model procedure of SAS software [19] with fixed effects of treatment and random effects of lamb nested in treatments:

$$Y_{ik} = \mu + D_i + L_k(D_i) + \epsilon_{ik} \quad (1)$$

where Y_{ij} = dependent variable; μ = mean; D_i = fixed effect of dietary treatment i ; $L_k(D_i)$ = effect of lamb k nested in the dietary treatment; ϵ_{ik} = error.

For repeated measure date model:

$$Y_{ijk} = \mu + D_i + \text{time}_j + D_i \times \text{time}_j + L_k(D_i) + \epsilon_{ijk} \quad (2)$$

where time_j = effect of time j as a fixed effect.

Measurements obtained before administration of dietary treatments were used as covariates. The covariates were removed from the model one at a time, starting with the least significant. Least square means, standard error of means, and P-values are reported. Statistical differences were considered significant when $P < 0.05$ and trends are discussed when $P < 0.01$.

3. Results and discussion

Kurdish ewe is the most popular indigenous dairy breed of sheep in the west of Iran. Its main characteristics are high prolificacy and high milk yield. Considering the high genetic potential of Kurdish sheep, it is important to ensure that appropriate management practices are implemented in these intensive production systems.

3.1 Intake, growth, and puberty

Discussing about the topic of sheep and lamb management over the last 40–50 years traditionally involved sheep management, growth development, and early weaning. In the last 10–20 years, the concept of “intensified feeding or accelerated growth” has become a focus of discussion, and during that time the concept has been applied to research programs and on farm in various ways.

The results of intake and BW that were obtained from ewe lambs pre- and post-weaning are summarized in **Table 2**. Results showed that initial BW was similar between all experimental groups ($P > 0.05$), but ewe lambs fed with HQD would gain faster than ewe lambs fed with LQD in BW. And also accelerated BW during the prepubertal period was achieved in the current study affecting the age of puberty (**Table 3**); this is in agreement with the results of Rosales et al. [20] in ewe lambs. BW includes muscle and fat and thus questions interpretation. An important consideration is that BW per se is simply mass and so encompasses physiological or mechanistic process that would affect the reproductive system [21]. In 2009 and 2010, Kenyon et al. indicated that there is a clear positive relationship between BW and reproductive performance in ewe lambs that aligns with the results of present studies.

Results revealed that the HQD treatment increased DMI, compared with the LQD treatment during pre-weaning period ($P < 0.01$). DMI in L-H group was lower than in H-H group, which is similar to the observations by Aguerre et al. [22]. DMI in H-L group was greater than in L-L group, lambs fed with the HQD in pre-weaning had greater DMI when they were fed with the LQD in post-weaning, and it seems that the increase in DMI led to larger body size. Ewe lambs from the H-L treatments also experienced reduced growth rates during post-weaning period, possible reasons for this result may be the larger body size, higher basal metabolism, higher energy, and protein requirements; with regard to rumen capacity and appetite of lambs, the LQD could not cover their needs at period post-weaning. Animals of L-H treatment could respond to diet quality changes but with lower rates than before weaning. Feeding LQD reduces the weight gain of growing animals and can result in greater growth rate once dietary conditions improve, and current results are in agreement with those reported by Drouillard et al. (1991).

Ewe lamb fresh milk intake and milk ME and CP intake were not affected by diet quality among treatments ($P > 0.05$, **Table 2**). During the post-weaning period, lambs of H-H treatment had higher ($P < 0.01$; **Table 2**) DMI, ME, and CP intake compared with other lamb treatments ($P > 0.05$).

The rates of ADG are shown in **Table 2**, indicating differences in the timing of responses to diet quality. Hence, responses to HQD were much greater at a younger

Item	Pre-weaning treatments		Item	Post-weaning treatments			
	HQD	LQD		HQD		LQD	
				H-H	H-L	L-H	L-L
n	20	20	n	10	10	10	10
Intake				Intake			
DM (kg/d)	0.97	0.64	DM (kg/day)	1.54	1.21	1.31	0.87
Fresh milk (kg/d)	1.11	1.18	ME (Mcal/day)	3.85	2.42	3.27	1.76
ME (diet + milk, Mcal/d)	3.49	2.44	CP (g/day)	228	104	194	76
CP (diet + milk, g/d)	1874	103	Puberty age (day)	123	254	168	267
BW				BW			
30 days (kg)	10.1	10.2					
120 days (kg)	31.2	22.5	210 days (kg)	43.8	33.9	36.3	26.6
ADG 30–120 days (g/day)	235	136	ADG 121–210 days (g/day)	138	31	153	57
FCR (%)	4.13	4.69	FCR (%)	11.1	38.7	8.57	15.26
<i>Pre-weaning treatments: HQD, high-quality diet; LQD, low-quality diet. Post-weaning treatments: H-H, HQD pre- and post-weaning; H-L, HQD pre-weaning and LQD post-weaning; L-H, LQD pre-weaning and HQD post-weaning; L-L, LQD pre- and post-weaning (control). DM, dry matter; ME, metabolite energy; CP, crude protein; BW, body weight; ADG, average daily gain; and FCR, feed conversion ratio.</i>							

Table 2.
Effects of pre- and post-weaning diet quality on intake and body weight of ewe lambs (30–210 days of age).

age, while responses to HQD were greater for ewe lambs that fed with LQD at pre-weaning and then received HQD at post-weaning, which is in agreement with other studies on sheep and cattle [23–25]. These authors indicate that increased severity of feed restriction is likely to increase the rate of growth after realimentation. Animals fed with HQD had higher BW and ADG compared with animals fed with LQD at pre-weaning period ($P < 0.01$). Lambs on the L-H group during the post-weaning had higher ADG than lambs on H-L and L-L groups ($P < 0.01$). However, lambs had higher ADG than H-H group, but this difference was not significant (153 vs. 138 g/day).

Growth rate and feed conversion ratio (FCR) are considered key production parameters by sheep farmers. Optimizing these parameters can better farm income by improving the production of the farm and/or by improving production efficiency (lambs produced per unit of feed consumed). Lambs consuming the HQD in pre-weaning had similar FCR with lambs fed with the LQD. Ewe lambs consuming the HQD in pre-weaning and LQD in post-weaning (H-L group) had greater FCR than other groups (**Table 2**). Ewe lambs in L-H group had lowest FCR and greater feed efficiency than H-H group.

Nutrition is a factor that influences the start of lamb’s puberty and has an important effect on sexual maturity [9]. Most Kurdish ewe lambs achieved puberty by 210–240 days of age when their average live weight was approximately 35 kg or approximately 65% of their estimated mature live weight [26]. The average puberty age of H-H and L-H groups was lower than H-L and L-L groups, respectively ($P < 0.05$, **Table 2**). Based on the results of current study, ewe lambs with higher growth rate were more likely to achieve puberty. And also these lambs were heavier at weaning time and grew faster during the post-weaning period. The ewe lambs of H-L group were heavier than ewe lambs of L-L group at weaning time and

post-weaning period. These results are consistent with previous reports that faster growth results in more ewes achieving puberty at a younger age in female sheep [20]. Most of the ewe lambs of L-L group did not show puberty at 210 days of age, and therefore they were removed from the first reproduction table. The average puberty age of H-H sequence was lower than other treatments, with no significant interaction of pre- and post-weaning ($P < 0.05$).

3.2 Hormones

The mean of serum insulin and leptin concentrations of ewe lambs at pre- and post-weaning is shown in **Table 3**. Serum insulin concentrations was higher for lambs fed with HQD compared with lambs fed with LQD (4.45 vs. 2.09 μ IU/ml, respectively, **Table 3**). Animals in H-H treatments had higher serum insulin concentration at 210 days of age compared with other treatments, while leptin concentration of ewe lambs of L-H group was higher than in other treatments at 210 days of age. These results indicated that maybe increasing weight gain at post-weaning contributed with higher fat fraction and also ultrasonography evidence from fat and muscle diameter of between 12- and 13-rib area support these results (the results were not reported).

Little is known about the importance of leptin in the early postnatal period, despite its potential role in important processes such as mammary gland and appetite regulation and a variety of other effects in the body [21]. Leptin is synthesized and secreted primarily particularly in adipose tissue, in addition to multiple other sites of production including the mammary gland and leptin regulated by multiple hormones including somatotropin, insulin, and IGF-I [27]. Leptin concentration was positively associated with higher values for growth and fat accumulation and therefore with an improvement in reproductive performance [20]. The current results support this concept because leptin concentration was positively correlated with body fat; however, endocrine links to the reproductive control centers have not been clearly identified.

The hormones insulin and leptin have primary roles in the control of reproduction in sheep. In times of decreased feeding, these hormones interact at the hypothalamus to reduce reproduction and enhance feeding [1]. Thus, nutrient reprioritization occurs in part at the expense of reproductive function as a survival mechanism. For example, reduced insulin occurs to spare glucose for central nervous system function, and reduced leptin occurs to allow stimulation of neuropeptide Y to in turn increase appetite as well as changes in anabolic hormones and,

Item	Pre-weaning treatments		Post-weaning treatments			
	HQD	LQD	HQD		LQD	
			H-H	H-L	L-H	L-L
n	20	20	10	10	10	10
Insulin (μ IU/ml)	4.45	2.09	4.48	2.32	3.59	1.35
Leptin (ng/ml)	2.84	2.20	3.03	2.06	3.66	1.98
Progesterone (ng/ml)	1.38	0.76	2.98	1.85	2.42	0.95

Pre-weaning treatments: HQD, high-quality diet; LQD, low-quality diet. Post-weaning treatments: H-H, HQD pre- and post-weaning; H-L, HQD pre-weaning and LQD post-weaning; L-H, LQD pre-weaning and HQD post-weaning; L-L, LQD pre- and post-weaning (control).

Table 3.
Effects of pre- and post-weaning diet quality on serum insulin and leptin concentrations of ewe lambs (30–210 days of age).

Item	Pre-weaning treatments		Post-weaning treatments			
	HQD	LQD	HQD		LQD	
			H-H	H-L	L-H	L-L
n	20	20	10	10	10	10
Tail measures (cm)						
Tail length	30.95	27.15	38.00 ^a	33.10 ^b	36.00 ^{ab}	29.26 ^c
Tail width	27.75	24.55	35.45 ^a	31.34 ^b	32.37 ^{ab}	27.18 ^c
Tail circumference	56.75 ^a	47.45 ^b	67.24 ^a	55.82 ^b	59.16 ^b	49.52 ^c
^{a,b,c} Means with distinct subscripts in the same row differ ($P < 0.05$). Pre-weaning treatments: HQD, high-quality diet; LQD, low-quality diet. Post-weaning treatments: H-H, HQD pre- and post-weaning; H-L, HQD pre-weaning and LQD post-weaning; L-H, LQD pre-weaning and HQD post-weaning; L-L, LQD pre- and post-weaning (control).						

Table 4.
Effects of pre- and post-weaning diet quality on tail measures of ewe lambs (30–210 days of age).

ultimately, reduce Kp and GnRH until feed is more readily available [21]. Rosales et al. [20] reported that leptin levels positively correlated with earlier puberty onset in ewe lambs. And also these authors reported that leptin concentration was not related to age at first estrus, but puberty and BW at first estrus were positively correlated with leptin concentration [20, 28, 29].

Progesterone can be used to monitor the pregnancy status and timing of puberty [30]. Within the post-weaning, serum progesterone concentrations were greater for ewe lambs fed at H-H group than for those fed the L-H, H-L, and L-L treatments, respectively ($P < 0.05$, **Table 3**). In conclusion, it was concluded that prepubertal plan plays an important role in secretion of progesterone, which leads to early sexual puberty. The present findings demonstrate that in the female lamb, LQD may impair the systems governing the luteinizing hormone that controls follicle growth and cycle hormone (progesterone secretion) and delays puberty.

3.3 Tail measures

At the 120 days of age, HQD lambs had higher tail circumference ($P < 0.01$; **Table 4**), than LQD lambs. At 210 days of age, after a period of feed restriction of H-L treatment, the average tail length, tail width, and tail circumference of lambs in the H-L group were lower than H-H lambs ($P < 0.05$; **Table 4**) but showed no difference compared with that of the L-H group ($P > 0.05$; **Table 4**). Animal growth has been defined as the net accretion of protein and fat in respective tissues, controlled by nutrition, environment, and genetic capacity to grow [31]. Hamouda and Atti [32] revealed that in young lambs, carcass adiposity and particularly fat tail reduce Barbarine meat value as the lamb grows. And also, fat stored in the body is an important energy source when food is rare. In the present work, results showed that better diet with greater energy and protein complementation was able to improve intake and growth performance.

4. Conclusions

The present study confirmed that a HQD improved the BW and ADG at weaning and breeding time. Based on the results of this research, weaning weight, previous nutrition plan, and current nutrition level are factors that determine puberty age, hormone concentration, and tail measures. To conclude, it was concluded that

prepubertal plan plays an important role in secretion of progesterone, insulin, and leptin, which can lead to early sexual puberty. And also these strategic plans should improve economic traits at the start of lamb's puberty in sheep husbandry.

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Author details

Sedigheh Menatian^{1*}, Hamidreza Mirzaei Alamouti², Farshid Fatahnia¹ and Reza Masoumi²

1 Department of Animal Science, Faculty of Agriculture, Ilam University, Ilam, Iran

2 Department of Animal Science, Faculty of Agriculture, Zanjan University, Zanjan, Iran

*Address all correspondence to: menatian@alumni.znu.ac.ir

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