Effects of pre-pubertal nutrition plan on blood metabolites and some physiological responses in Kurdish female lambs at weaning and breeding

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Abstract:

BACKGROUND: Pre-pubertal nutrition managements are considered essential for animal health, optimizing production and reproductive performance in livestock. **OBJECTIVES:** In this study, the effects of dietary low and high energy and protein provisions on body growth, blood metabolites and inherent safety index were investigated in pre-pubertal Kurdish female lambs. **METHODS:** We fed 40 healthy Kurdish female lambs (30±8.6 days old and weighing 10.2±3.4 kg) were randomly divided into one of two experimental diets in pre-weaning period: high energy and protein (HEP, 2.50 Mcal ME/kg dry matter (DM) and 148 g CP/kg DM) or low energy and protein (LEP, 2.02 Mcal ME/kg DM and 87 g CP/kg DM). At the time of weaning, one half of lambs from each group was randomly separated and assigned to HEP or LEP. So there were four treatment groups in post-weaning period: H-H (HEP pre- and post-weaning); H-L (HEP pre-weaning and LEP post-weaning); L-H (LEP pre-weaning and HEP post-weaning) and L-L (LEP pre and post-weaning, control group). Treatment effects on dry matter intake, milk intake, serum metabolite profiles, inherent safety index and growth were examined. **RESULTS:** Pre-pubertal plane were significantly affected above mention parameters at post- weaning period ($p<0.05$). In the post-weaning period, dietary treatment did significantly decrease affect all blood metabolites concentration ($p<0.05$) expect calcium levels. The numbers of white blood cell, neutrophil, lymphocytes and monocytes of H-L treatment were higher than other groups ($p<0.05$). **CONCLUSIONS:** It has been observed that diet energy and protein and body energy reserves are much important regulators of growth and health indicators in Kurdish female lambs.

Introduction

In recent years, research has focused on direct or indirect links between nutrition and growth (Daniel et al., 2013). During previous decades, in farm animals, just as in humans, there is increasing evidence that production and growth characteristics in farm animals may be affected by pre-pubertal plane of nutrition (Menatian et al., 2016). Nutritional management is considered essential for optimizing production and reproductive performance in livestock (Daniel et al., 2013).

In tropical, semi-arid, and arid areas, animal production is dependent on supplemental feeding, especially in the reproductive seasons due to the higher energy demand (Blache et al., 2008). In the west of Iran sheep have been proved to perform well under extensive, semi-intensive as well as intensive management systems. In these climates it is often difficult to meet these requirements because quantity and quality of forage varies with season, and can be scarce during dry-seasons. Therefore, they are economically important mainly on account of their short gestation period, generation interval and high prolificacy (Danial et al., 2013). The natural resistance and adaptive capabilities of animals are challenged under the constant influence of various factors, some of which are stress-inducing. One of the most significant external factors is feeding, and its characteristics type and quality, ratio of the various nutrients, diet balancing with regard to protein and carbohydrates and other nutrients (Danial et al., 2013). Under the influence of stressors, the body reacts by activating neurohormonal regulatory mechanisms, through which it maintains the homeostasis, the objective assessment of which are the blood indicators (Kaneko, 1989). The physiological stress of sheep can be indicated by the concentration of blood constituents, such as glucose, plasma urea N (Krawczel et al., 2007), and numbers of white blood cells and platelets (Zhong et al., 2011).

There are a few lambs' studies that investigated the effects of dietary low-high carbohydrate and protein intake during pre-pubertal on body growth, blood metabolites and inherent safety index. Therefore, the main aim of the present study was to determine whether dietary low and high energy and protein levels can affect body growth, blood metabolites and inherent safety index in pre-pubertal Kurdish ewe lambs in Zagros Mountains west of Iran.

Materials and Methods

Experiments were conducted at Nomadic Management Department, Ilam Province, Iran (33º51´N, 46º 27´E) from January 2013 to December 2015.

A total of 40 healthy (numbers of animals for per treatment=10) Kurdish female lambs (30± 8.6 days old and weighing 10.2 ± 3.4 kg) were used. At 30 days of age, lambs were randomly housed together and had twice daily access to their mother's milk and were allocated to one of two experimental treatments to achieve either high or low rates of body weight (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 3 consecutive days every 2 weeks for recording dry matter intake (DMI). In pre-weaning period the lambs were fed high energy and protein diet (HEP, n = 20) or low energy and protein diet (LEP, n = 20) and at the weaning time HEP and LEP fed lambs were re-randomized. So that one half of lambs from each group was randomly allocated to HEP or LEP. So there were four treatment groups (n = 10) in post-weaning period: HEP pre- and post-weaning (H-H); HEP pre- weaning and LEP post- weaning (H-L); LEP pre-weaning and HEP post- weaning (L-H), and LEP pre- and post- weaning (L-L, control group). The HEP and LEP were formulated according to nutrient requirements for small ruminants (NRC, 2007) recommendations that received a diet that covered nutrient requirements including energy and protein needs for a 20-kg growing lamb with an average daily gain of 200 and 100 g/d, respectively. Diets were formulated to have different metabolized energy (ME) and crude protein (CP) content. The HEP and LEP 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. Ingredient and chemical composition of the experimental diets are shown in Table 1.

Sampling and recorded parameters: The BW were 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 mix ration and orts were determined to estimate DMI. ME and CP intake were calculated as DMI from each diet multiplied by their ME and CP contents, respectively (NRC, 2007). DM, CP and ether extract (EE) of experimental diets were measured according to the methods of AOAC (1995). The neutral detergent fiber (NDF) was measured according to the method described by Van Soest et al., (1991) without α-amylase and sodium sulfite and was expressed exclusive of residual ash. Non-fibrous carbohydrates (NFC) content was calculated according to NRC (2001) dairy cattle model as 100 - (CP+NDF+EE+ash). Milk intake by ewe lambs was measured by the weigh-suckle-weigh method (WSW) (Doney et al., 1979) in 3 consecutive days every 2 weeks from the start of study to weaning (30-120 d). 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. On each milking occasion, ewes were milked by hand after intravenous injection of 1 IU synthetic oxytocin (Van der Linden et al., 2010). Milk samples of dams in subsequent lactation were collected in 3 consecutive days every 2 weeks and analyzed for fat, protein and lactose by Milk-O-Scan 133B (Foss Electric, Hillerod, 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 GE = ((0.0547 × CP %) + (0.0929 × Fat %) + (0.0395 × Lactose %)) according to NRC (2001). The mean metabolize ability of the ewe milk GE is 0.94 (Treacher and Caja, 2002), therefore, milk ME content was calculated as GE × 0.94. Energy corrected milk (ECM) and fat corrected milk (6.5% FCM) were calculated as ECM = (0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein) and FCM = Milk yield × (0.37 + (0.097 × Fat %))(Tyrrel and Reeds, 1965).

Blood samples (5 ml) were collected in K3EDTA coated tubes and then centrifuged (3000×g, 15 min, 4°C) within 1 h after the collection of the sample to separate the serum, sera were separated into 1.5 ml micro tubes and then placed in freezer (-20 °C). Serum samples were tested for blood metabolites by Spectrophotometric method and commercially available kits (Pars Azmon, Karaj, Iran) were purchased from Intervet Drug Industry (Tehran, Iran). Whole blood was used to measure white blood cell

(WBC), red blood cell (RBC), and numbers of platelets (PLT), neutrophils (NEU), lymphocytes (LYM), monocytes and hemoglobin concentration using an automatic blood cell analyzer (Coulter LH 750, Beckman Coulter).

The data sets obtained were analyzed as a completely randomized 2 × 2 factorial design, using the mixed model procedure of the SAS software (SAS Inst. Inc., Cary, NC, 2003), with fixed effects of treatment and random effects of lamb nested in treatments.

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

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 data, the model was:

(2) $Y_{ijk}=\mu+D_i+Time_j+D_i\times Time_j+Lk(D_i)+ \varepsilon_{ijk}$

$Time_j$ = effect of time j as a fixed effect

Initial weight and birth weight were used as covariates in the model. The covariates were removed from the model one at a time, starting with the least significant. LSM, SEM and P-values are reported. Statistical differences were considered significant when p<0.05 and trends are discussed when p<0.01.

Results

Weights during 30-120 days pre-weaning and 120-210 days post-weaning were significantly affected by pre-pubertal plane (p<0.05; Table 2 and 3). Pre-pubertal plane affects dry matter intake during the pre-weaning period (p<0.05; Table 2). At 120 days of age, HEP lambs had higher tail circumference (p<0.01; Table 2), than LEP lambs. At 210 days of age, after a period of feed restriction of H-L treatment, the average BW, DMI, tail length, tail width and tail circumference of lambs in the H-L group were lower than H-H lambs (p<0.05; Table 3), but showed no difference compared with that of the L-H group (p>0.05; Table 3).

The least square means for blood metabolites are presented in Table 4 and 5. In the pre-weaning period, dietary treatment had no effect (p>0.05) on blood metabolites concentration except glucose (HEP=68.82 vs. LEP=60.70 mg/dL) and BUN (HEP=15.07 vs. LEP=10.14 mg/dL) levels. In the post-weaning period, dietary treatment significantly decreased all blood metabolites concentration (p<0.05) except calcium levels. Animals of HL treatment

Table 1. Main ingredient and chemical composition of experimental diets. Pre-post weaning treatments: HEP = High Energy and Protein Diet; LEP = Low Energy and Protein 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 = Non-Fiber Carbohydrates = 100 - (CP+NDF+EE+ ash); ME = Metabolizable Energy.

Composition (%)	Pre-post-weaning diets	
	HEP	LEP
Ingredient		
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
Chemical		
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

Table 2. Effect of pre-weaning plan of nutrition on growth, diets intake and milk intake in Kurdish female lambs. Pre-weaning treatments: HEP = High Energy and Protein Diet; LEP: High Energy and Protein Diet; SEM: Standard Error of Mean. ADG: Average Daily Gain; DMI: Dry Matter Intake; MEI: Metabolite Energy Intake; CPI: Crude Protein Intake; FMI: Fresh Milk Intake; ECM: Energy Corrected Milk ((0.327 × kg milk yield) + (12.95 × kg fat) + (7.2 × kg protein)); CP: Crude Protein.

Parameters	Pre-weaning treatments		SEM	p-value
	HEP	LEP		
Weight (kg)				
30	10.04	10.20	1.12	0.21
120	31.20a	22.50b	0.73	<0.01
ADG 30-120 (gr/d)	235a	136b	24	<001
Tail measures (cm)				
Tail length	30.95	27.15	1.38	0.06
Tail width	27.75	24.55	1.18	0.06
Tail circumference	56.75a	47.45b	1.97	<0.01
Intake				
DMI (kg/d)	0.97a	0.64b	0.03	<0.01
MEI (Diet+ Milk, Mcal/d)	3.49a	2.44b	0.18	<0.01
CPI (Diet+ Milk, g/d)	187.4a	103b	6	<0.01
FMI (kg/d)	1.11	1.18	0.10	0.33
Milk composition				
ECM	0.973	0.974	0.035	0.98
CP	4	4.02	0.05	0.81

Table 3. Effect of post-weaning plan of nutrition on growth and diets intake in Kurdish female lambs. Pre-weaning treatments: HEP = High Energy and Protein Diet; LEP = Low Energy and Protein Diet; Post-weaning treatments: H-H = HEP pre and post-weaning; H-L = HEP pre-weaning and LEP post-weaning; L-H = LEP pre-weaning and HEP post-weaning; L-L = LEP pre and post-weaning (control). ADG: Average Daily Gain; DMI: Dry Matter Intake; MEI: Metabolite Energy Intake; CPI: Crude Protein Intake. [a,b,c] Means in rows with no common superscripts differ (p<0.05).

Item	Post-weaning treatments			
	HEP		LEP	
	H-H	H-L	L-H	L-L
Number of lambs	10	10	10	10
Weight				
ADG 120-210 (gr/d)	138 a	31 b	153 a	57 b
210 (kg)	43.7 a	33.8 b	36.4 b	26.6 c
Tail measures (cm)				
Tail length	38.00a	33.10b	36.00ab	29.26c
Tail width	35.45a	31.34b	32.37ab	27.18c
Tail circumference	67.24a	55.82b	59.16b	49.52c
Intake				
DMI (kg/d)	1.54 a	1.20 b	1.31 b	0.87 c
MEI (Mcal/d)	3.85 a	2.42 c	3.27 b	1.76 d
CPI (g/d)	228 a	104 c	194 b	76 d

during the post weaning had lower plasma concentrations of total protein, albumin and BUN (p<0.05).

The least square means for inherent safety index are presented in Table 4 and 5. In the pre-weaning period, the numbers of PLT (HEP=123.42 vs. LEP=128.73 × 10_9/L) were influenced (p<0.05) by dietary treatment.

Table 4. Effect of pre-weaning plan of nutrition on blood metabolites and inherent safety index in Kurdish female lambs. Pre-weaning treatments: HEP = High Energy and Protein Diet; LEP: Low Energy and Protein Diet; SEM: Standard Error of Mean.

Metabolites	Pre-weaning treatments		SEM	p-value
	HEP	LEP		
Glucose (mg/dL)	68.82a	60.70b	1.02	<0.01
Total protein (g/dL)	5.94	5.78	0.17	0.54
Albumin (g/dL)	3.53	3.51	0.08	0.83
BUN (mg/dL)	15.07a	10.14b	0.59	<0.01
Calcium (mg/dL)	8.51	9.41	0.85	0.45
Phosphorus (mg/dL)	5.79	5.69	0.13	0.58
Log WBC (×109/L)	7.01	7.13	1.17	0.23
Log RBC (×1012/L)	4.29	4.47	1.09	0.34
Log platelets (×109/L)	123.42b	128.73a	1.02	0.03
Neutrophils (%)	12.54	11.37	0.86	0.18
Lymphocytes (%)	48.42	49.55	1.13	0.55
Neutrophils : Lymphocytes	0.26	0.23	0.02	0.12
Monocytes (%)	8.68	7.93	0.75	0.69
Hemoglobin (g/L)	71.62	72.24	0.62	0.41

Table 5. Effect of post-weaning plan of nutrition on blood metabolites and inherent safety index in Kurdish female lambs. Pre-weaning treatments: HEP = High Energy and Protein Diet; LEP = Low Energy and Protein Diet; Post-weaning treatments: H-H = HEP pre and post-weaning; H-L = HEP pre-weaning and LEP post-weaning; L-H = LEP pre-weaning and HEP post-weaning; L-L = LEP pre and post-weaning (control). [a,b,c] Means in rows with no common superscripts differ ($p<0.05$).

Metabolites	Post-weaning treatments			
	HEP		LEP	
	H-H	H-L	L-H	L-L
Glucose (mg/dL)	74.46 a	62.55 b	73.83 a	67.27 b
Total protein (g/dL)	6.91 a	5.53 b	6.69 a	6.32 a
Albumin (g/dL)	3.87 a	2.44 c	3.51 ab	3.23 b
BUN (mg/dL)	19.53 a	10.76 c	17.07 ab	12.14 bc
Calcium (mg/dL)	9.26	8.68	9.9	9.09
Phosphorus (mg/dL)	5.67 a	5.45 ab	5.36 ab	4.96 b
Log WBC (×109/L)	6.57b	9.65a	6.03b	5.82b
Log RBC (×1012/L)	5.32	5.73	5.56	6.12
Log platelets (×109/L)	109.03	112.32	103.83	125.47
Neutrophils (%)	9.33b	14.45a	8.07b	7.65b
Lymphocytes (%)	52.48b	60.09a	54.11b	55.17b
Neutrophils : Lymphocytes	0.18b	0.24a	0.15b	0.14b
Monocytes (%)	8.45b	22.03a	9.34b	9.21b
Hemoglobin (g/L)	76.09	71.68	74.82	75.51

In the post-weaning period, the number of WBC in H-L group was greater ($P<0.05$) than other groups. Animals in H-L group had higher ($p<0.05$) NEU: LYM ratio, percentages of NEU, LYM and monocytes compared with other treatments.

Discussion

Accelerating the growth of sheep has the potential to increase profitability by reducing the time interval from birth to first lambing, subsequently reducing feed, labor, housing, and other costs associated with raising replacement animals.

In sheep husbandry, feeding fiber-protein-enriched diets to lambs is attracting interest from economic and social perspectives. The objective of the current study was to determine whether dietary low-high carbohydrate and protein can affect body growth, blood metabolites and inherent safety index in pre-pubertal lambs in Zagros Mountains west of Iran. Those affecting lamb growth, blood metabolites and inherent safety index are still largely unknown. To our knowledge, this experiment is the first to report on the response to dietary supplementation of low-high carbohydrate and protein in Kurdish female lambs during pre-pubertal period.

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 (MacGhee et al., 2016). Hamouda and Atti (2011) revealed that in young lambs, carcass adiposity and particularly fat tail reduces Barbarine meat value as the lamb grows. And also, fat stored in the body is an important energy source when food is scarce. In the present work, results showed that better diet with greater energy and protein complementation was able to improve intake and growth performance. In line with the results of present study, Mohana Devi et al. (2014) reported that supplementation of protein sources in growing pig diets improved the growth rate and feed intake. Based on the results of the present study proper growth rate increased the profitability at farm le vel in thewest of Iran and this can only be achieved by identifying the specific associated factors such as proper nutrition. Our results also demonstrate that energy and protein of diet is a determining factor in weight improvement and growth development. Growth rate is related to rate of maturing and mature weight and these latter traits have been suggested to have a relationship with other female lifetime productivity parameters in lambs.

In a study carried out by Yang et al (2014) and Shevah et al., (1975) it was reported that animals raised on a restricted feed intake show growth retardation, greater GH secretion and greater non-esterified free fatty acids (NEFA). Therefore, increased plasma NEFA concentrations may be indicative of increased stress in small ruminants (Slimen et al., 2015). Results revealed that decreasing the dietary energy and protein levels in HL group, led to a significant reduction in body fat reserves and all of the tail measures. Lambs on the HL and LL treatments failed to attain the target growth rate of 100 g/day in second rearing period.

Our results showed that decreasing the dietary energy and protein levels led to a significant reduction in important blood metabolites in lambs. The plasma glucose concentration was affected by dietary treatments, which is in agreement with several previous studies (Ehtesham and Vakili, 2015; Dong Hyeon et al., 2016). Brickell et al., (2009) suggested that plasma urea is not only a marker of protein intake among different diets, but also an indirect marker of DMI. BUN concentrations were affected by dietary treatments. This may be because DMI and CP digestibility were affected by diet in that study. We utilized soybean meal in HEP ration and this feed has high levels of rumen degradable protein (RDP) and degraded by ruminal bacteria (NRC, 2001). The lack of an interaction between blood metabolites may have reflected the use of the relatively low level of fiber, the high level of nutrients in the diet, and the clean environmental status of the trial farm.

Our results showed that feed restriction after a period of abundance of feed (H-L treatment) increased numbers of white blood cells (NUT, LYM and monocytes). The observed changes (leukocytosis) are typical for the so-called "stress leukogramme", which is due to the increased endogenous production of cortisol from the adrenal glands, following the action of stress factors, one of which is feed restriction (Chung et al., 2001). Stress-inducing factors cause an increase in the production of catecholamines in blood, as a result of adrenal gland hyperfunction and a simultaneous inhibition of the production of insulin in the pancreas (Shehab et al., 2012) and caused change in immune system response (Chung et al., 2001). It is also possible that an activation of the immune system, caused by inflammation, could increase numbers of WBC (Rosales et al., 2016).

Conclusion: Ours findings suggest that improved body weight and growth rate of Kurdish lambs may be achieved by increasing carbohydrate and protein intake at pre-pubertal gain.

And also, these findings suggest that reducing diet energy and protein in animals with good body condition may play a role in the severe activation of the immune system.

Finally, these findings can help to identify times at which changes in management may improve the lamb performances and to develop appropriates breeding practices in sheep husbandry.

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