



### ABSTRACT

This experiment was conducted to investigate the effect of pre-pubertal plane of nutrition on the skeletal growth, lamb mortality, insulin-like growth factor 1 (IGF-1) concentrations, quantity and quality of colostrum produced 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 preand 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). Weekly DM intake was determined. Serum IGF-1 concentrations was determined by ELISA method. The HQD treatment increased DM intake and BW (body Weight) compared with the LQD treatment during pre-weaning period (P<0.01). At 210 d of age, animals fed LQD during the pre-weaning period and HQD during the post-weaning period (L-H sequence) had greater body length (BL), wither height (WH), hip height (HH) and hip width (HW) than animals on the H-L sequence. No interaction of the two periods was detected for heart girth, HW, WH, BL at 210 d of age (P>0.05). HW at weaning time was not affected by quality of diet. However, lambs of H-H and L-H sequences had a higher increase in HW during post-weaning period compared with lambs of H-L and L-L sequences. Quantity and quality of colostrum was not influenced by pre-pubertal plane of nutrition (P>0.05). Based on the results of current study the authors' suggestion is that skeletal compensatory growth during post-weaning period can compensate losses caused by poor nutrition during pre-weaning period. And also, the results indicated that skeletal size is a better indication for first-lactation colostrum yield than body weight (BW).

KEY WORDS colostrum, IGF-1, Kurdish ewes, lamb mortality, pre-pubertal diets, skeletal growth.

# INTRODUCTION

Small ruminants are a common of many traditional farming systems near the Zagros Mountains in the west of Iran. Despite advances in animal science husbandry during previous decades, selling lambs are the main source of incomes for small holder in this area. Profitability in sheep husbandry in Iran is closely related to the ability of breeding ewes to raise lambs at the maximum economically feasible level. One approach to economic feasibility sheep production in a closed space is manipulating the age of puberty. In order to accelerate the maturity of the ewe lamb, the traditional plane of nutrition must be changed. However, due to the high positive correlation between BW and lamb mortality it is necessary to apply the right strategies. All of factors that affect the rate of growth pre- and post-weaning are important determinants of age at puberty. If a ewe lamb fails to achieve puberty in its first autumn, it will be delayed until the following breeding season (Kenyon *et al.* 2014).

Studies on animals revealed that both prenatal and neonatal programming of skeletal system development may be induced with the use of nutritional manipulation (Harrison *et al.* 2004; Tatara *et al.* 2007; Andersen *et al.* 2008). This suggests that the opportunity for increasing skeletal growth rate is greater during the time prior to puberty. Body weight fails to indicate the composition of the animal, therefore, measurements of the animal's frame can be considered indirect indicators in determining meat leanness (Greyling and Taylor, 1999).

Abnormal or difficulty in giving birth can be led to lamb mortality. There are two types of factors that lead to incidence of dystocia in ewe. Firstly, the fetal factors which include oversized fetus, lamb malpresentation, malposition, postural defects, and congenital abnormalities. Secondly, the maternal factors which include over feeding of dam during pregnancy, uterine inertia in polytocous ewes, and small diameter of pelvic canal (Pugh and Baird, 2012).

After a safe parturition, another challenge for livestock producer is to produce healthy newborn lambs. Ruminant neonates rely entirely on colostrum and milk from their dam for survival (Stelwagen *et al.* 2009). Newborn ruminants require a sufficient amount of colostrum within 48 h post-partum to survive (Stelwagen *et al.* 2009). The colostrum contains an important antibody which provides a defense mechanism for newborn ruminants until their own immune system is established (Ahmad *et al.* 2000; Yilmaz and Kaşikçi, 2013). Furthermore, nutritional supplementation of dams can enhance the erythropoietic response and therefore improve offspring survival (Ahmad *et al.* 2000).

The objective of this study was to compare the effects of diet quality fed during the pre- and post-weaning periods and existence of potential interactions between pre- and post-weaning diets on serum IGF-1 concentrations, skeletal size, lamb mortality, quantity and quality of colostrums production in Kurdish ewe lambs.

### MATERIALS AND METHODS

#### Hormonal drugs

Controlled internal drug release (CIDR) with 300 mg of progesterone, a progestagen analogue (InterAg, Hamilton, New-Zealand), PMSG (folligon; Intervet International B.V., Boxmeer, the Netherlands), IGF-1 (LDN. Germany.

LOT:150702) and progesterone (DiaMetra. Italy. LOT N:4026) were used.

### Locations, animals and treatment schedule

This study was performed at Nomadic Management Department, Ilam Province, Iran  $(33^{\circ} 5' \text{ N}, 46^{\circ} 27' \text{ E})$  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 health Kurdish female lambs (30±8.6 d and weighing 10.2±3.4 kg) were used in this experiment.

At 30 d of age, lambs were randomly housed together with twice daily access to their mother milk and to one of two supplemental dietary treatments to achieve either high or low rates of BW gain during two consecutive periods of 30 to 120 (pre-weaning period) and from 121 to 210 d 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 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 rerandomized. So that one half of lambs from each group randomly allocated to HQD or LQD. So there were four treatment groups (n=10) in post-weaning period: HQD preand post-weaning (H-H); HQD pre- weaning and LQD postweaning (L-H) and LQD pre- and post- weaning (L-L, control group).

The HQD and LQD were formulated according to nutrient requirements for small ruminants (NRC, 2007) recommendations covered the energy and protein needs for a 20 kg growing lamb with an average daily gain of 200 and 100 g/d, respectively. The HQD and LQD 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. Ingredients and chemical composition of the experimental diets are shown in Table 1.

#### Estrous synchronization and pregnancy diagnosis

When ewe lambs reached 210-d-old, estrus was induced and synchronized by CIDR. Animals were treated with CIDR for 14 d 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 5 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 pasture until 2 weeks before expected parturition. Pregnancy diagnosis was determined by using of transabdominal ultrasound (Piemedical, Falco 100; Netherlands) at 60 d after serving.

Ingredients (%)	Pre- and post-weaning diets			
Ingredients (%)	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 <sup>1</sup>	39.6	46.4		
Composition				
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		

HQD: high quality diet; LQD: low quality diet; DM: dry matter; CP: crude protein; EE: ether extract; NFC: non-fiber carbohydrates and ME: metabolite energy. <sup>1</sup> Each kg (DM basis) of mineral and vitamin premix contained: Ca 180 g; P: 70 g;

K: 35 g; Na: 50 g; Cl: 58 g; Mg: 30 g; S: 32 g; Mn: 5 g; Fe: 4 g; Zn: 3 g; Cu: 300 mg; I: 100 mg; Co: 100 mg; Se: 20 mg; vitamin A: 400000 IU; vitamin  $D_3$ : 100000 IU and vitamin E: 245 IU.

### Data collection and calculation

The body weight (BW), body length (BL), heart girth (HG), wither height (WH), hip height (HH) and hip width (HW) were measured every 2 weeks from 30 to 210 d of age. WH and HH were measured by using of vertical graduated rod, BL, HG and HW by tape measure. BW was measured every 2 weeks from 30 to 210 d of age. Feed offered and feed refusals of individual pens were weighed and recorded daily and DM content of total mix ration (TMR) 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  $\alpha$ 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 weighsuckle-weigh method (WSW) 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. After lambing, ewe lambs were hand milked twice daily throughout lactation and milk yield was recorded at each milking for the entire lactation (two months). On each milking occasion, ewes were milked by hand after intravenous injection of 1 IU synthetic oxytocin. Milk samples of dams and ewe lambs in subsequent lactation were collected in 3 consecutive days every 2 weeks and analyzed for fat, protein and lactose by using of 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:  $GE=((0.0547 \times CP))$ %)+(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  $GE \times 0.94$ .

Energy corrected milk (ECM) and fat corrected milk (6.5% FCM) were calculated as ECM=  $(0.327 \times \text{kg milk}) + (12.95 \times \text{kg fat}) + (7.2 \times \text{kg protein})$  and FCM= milk yield ×  $(0.37+(0.097 \times \text{fat \%}))$ .

#### **Blood sampling**

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

#### Statistical analyses

The data of pre-weaning parameters were subjected to statistical analysis by using of completely randomized design (CRD). Data were analyzed as a CRD in factorial arrangement ( $2\times2$ ) by using of the mixed model procedure of SAS (2003) with fixed effects of treatment and random effects of lamb nested in treatments.

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

Where:

Y<sub>ij</sub>: dependent variable.

μ: mean.

D<sub>i</sub>: fixed effect of dietary treatment I.

 $L_k(D_i)$ : effect of lamb k nested in the dietary treatment.

 $\epsilon_{ik}$ : error.

For repeated measure data, the model was:

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

Where:

Time<sub>j</sub>: 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. 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 AND DISCUSSION**

Accelerating the growth of sheep has the potential to increase the profitability by reducing the time need from birth to first lambing, subsequently reducing feed, labor, housing, and other costs associated with raising replacement animals. Kurdish ewe is the most popular indigenous dairy breed of sheep in 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 their intensive production systems.

#### Intake and growth

Table 2 shows feed intake and skeletal growth (as measured by body weight (BW), body length (BL), heart girth (HG), wither height (WH), hip height (HH) and hip width (HW)) measurements by treatment between 30 and 210 d age. The HQD treatment increased DMI and BW compared with the LQD treatment during pre-weaning period (P<0.01). Lambs of H-H sequence had higher DMI, ME and CP compared with lambs of H-L, L-H and L-L treatments at postweaning period with no interaction of periods (P>0.05).

BL was increased by 39 and 31 cm by HQD and LQD treatments, respectively and HG was increased by 24 cm by HQD and 19 cm by LQD treatment, during pre-weaning period (P<0.01). At 120 d of age, ewe lambs fed the HQD treatment had greater WH and HH than lambs on the LQD treatment (P<0.01).

There were no differences among treatments in HW at weaning time. At 210 d of age, animals fed LQD during the pre-weaning period and HQD during the post-weaning period (L-H sequence) had greater BL, WH, HH and HW than animals on the H-L sequence. Also at the end of experiment, lambs on the L-H treatment had greater BL, HG, WH and HH than L-L sequence (P<0.01).

Ewe lambs on the H-H and L-H treatments were taller at 210 d of age than lambs on the H-L and L-L treatments (P<0.01). By comparing the pre- and post-weaning periods in the H-H sequence, BL increased by 35 cm during preweaning period, while it raised only 12 cm during postweaning period. A part from the HW, the trend was the same for other measured parameters. Pre-weaning skeletal growth rate was more than three times the growth rate post weaning. Some parameters like shoulder height and shoulder width grow at a slower rate than body length, but again these measurements had highly linear correlation with live body weight (Greyling and Taylor, 1999). Results of the present study indicated that the ewe lambs fed the HQD would gain faster than ewe lambs fed the LOD in both BW and skeletal size. Skeletal growth rate of H-L group was lower than L-L group during post-weaning period, but final skeletal size of H-L sequence was higher than L-L sequence, showing the importance of pre-weaning plane of nutrition. Our experiment results showed that more than 80% of pre-pubertal skeletal growth to maturity can be related to the pre-weaning period. With respect to higher skeletal growth rate, responses to diet quality will be dependent on several factors including the capacity of the animal for skeletal growth, the quality of the diet and protein with respect to meeting the animal's mineral and amino acid needs for increased bone synthesis. Mature skeletal size is determined by genetic potential, but quality of diet and feeding plane can result in animals achieving that genetic potential earlier or being retarded in growth (Owens et al. 1993). However, in some experiments (Radcliff et al. 1997; Van Amburgh et al. 1998) increased energy or a combination of energy and protein decreased skeletal size in ruminants. Since the rate of protein and mineral deposition decreases with age, the response will diminish as the animal matures. Based on the results of the current study the HQD diet was more effective during the early parts of the study than the mature lambs. As expected, we observed that that skeletal compensatory growth during post-weaning period can be compensating for losses caused by poor nutrition during this period. The most important functions of IGF-1 in relation to the skeletal system include its stimulating effect on longitudinal bone growth, proliferation and differentiation of chondrocytes in the growth plate, cortical bone formation, proliferation and differentiation of osteoblasts and type I collagen synthesis (Kanbur et al. 2005). Tatara (2008) was reported that the anabolic response of biochemical markers of bone formation at both these developmental stages of animals was analogous to the changes in serum concentration of IGF-1 induced by the pre-weaning treatment of lambs with HQD, great importance of somatotrophic axis function is in the regulation of bone metabolism and skeletal system development.

Item	Pre-weanir	ng 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/d)	1.54	1.21	1.31	0.87
Fresh milk intake (kg/d)	1.11	1.18	ME (Mcal/d)	3.85	2.42	3.27	1.76
ME intake (diet+milk, Mcal/d)	3.49	2.44	CP (g/d)	228	104	194	76
CP intake (diet+milk, g/d)	187.4	103	Puberty age (d)	123	245	168	267
Body weight			Body weight				
30 d (kg)	10.04	10.2	-	-	-	-	-
120 d (kg)	31.20	22.50	121-210 d (g/d)	138	31	153	57
30-120 d (g/d)	235	136	210 d (kg)	43.8	33.9	36.3	26.6
Body length			Body length				
30 d (cm)	93.7	90.6	-	-	-	-	-
120 d (cm)	132.9	122	121-210 d (cm/d)	0.13	0.003	0.19	0.03
30-120 d (mm/d)	4.4	3.5	210 d (cm)	145	133.2	138.9	124.8
Heart girth			Heart girth				
30 d (cm)	55.6	53.3	-	-	-	-	-
120 d (cm)	80	72.5	121-210 d (cm/d)	0.101	0.016	0.09	0.03
30-120 d (mm/d)	2.7	2.1	210 d (cm)	89.1	81.5	80.5	74.9
Wither height			Wither height				
30 d (cm)	44.3	43.9	-	-	-	-	-
120 d (cm)	59.4	55	121-210 d (cm/d)	0.07	0.03	0.11	0.04
30-120 d (mm/d)	1.67	1.2	210 d (cm)	66.5	63	65	59.2
Hip height			Hip height				
30 d (cm)	46.4	45.6	-	-	-	-	-
120 d (cm)	61.8	57.2	121-210 d (cm/d)	0.07	0.03	0.11	0.04
30-120 d (mm/d)	1.71	1.3	210 d (cm)	68.1	64.8	67.4	60.4
Hip width			Hip width				
30 d (cm)	16.8	15.8	-	-	-	-	-
120 d (cm)	22	21.8	121-210 d (cm/d)	0.06	0.02	0.05	0.01
30-120 d (mm/d)	0.6	0.6	210 d (cm)	27.5	23.9	26.2	22.8

 Table 2
 Effect of pre- and post-weaning plane of nutrition on intake and skeletal growth of ewe lambs (30-210 d of age)

HQD: high quality diet; LQD: low quality diet; H-H: HQD pre and post-weaning; H-L: HQD pre-weaning and LQD post-weaning; L-H: LQD pre-weaning and HQD postweaning and L-L: LQD pre and post-weaning (control).

DM: dry matter; CP: crude protein and ME: metabolite energy.

#### **Reproductive performance**

The L-L treatment was removed from the statistical analysis of reproduction performance, because of eight animals of this treatment were not pregnant. The rate of pregnancy H-H, H-L, L-H and L-L treatments were 100, 50, 70 and 20%, respectively (Table 3). Normal gestation length for sheep is between 144 and 152 days, and also according to results of Echternkamp and Gregory (1999), factors linked to gestation length were retained placenta, age of the dam, and sex of the lamb. There were differences in lamb birth weights between three groups (Table 3). Age at puberty, age at first lambing, safe and successful parturition at a younger age, stress, optimal nutrition, breed and geographical region are important traits concerning overall reproductive performance.

However, 3.6 kg by L-H sequence is relevant to 7 lambs and 2.7 and 2.8 kg by H-H and H-L groups are relevant to 10 and 5 lambs, respectively (Table 3). In general, at the mating time, animals that have sufficient weight, struggle less to reach their mature weight and bring the nutrients to the fetus. Lambs birth weight of H-H sequence was lower than in other treatments (Table 3). It seems that more than 80% of mature weight by the age of 7 months has a negative effect on lambs' birth weight. The incidence of lambs mortality was higher in L-H sequence (43%) compared with H-H (20%) and H-L (40%) treatments, which may be due to the higher birth weight of lambs of the treatment. In spite of good HW of dams, animals of H-H sequence showed dystocia and lower lambs' birth weight (Tables 2 and 3). Small pelvises in ewes are associated with high incidences of dystocia, high prenatal ewe and lamb mortality rates and poor lifetime rearing performance of ewes (Hartwig, 2002). High birth weights have been associated with increased dystocia in ewe bearing single lambs and young ewes are more susceptible to lambing problems than mature ewes that have lambed previously (Anderson, 1992; Hartwig, 2002). Growing animals on a low nutrient diet have clearly resulted in an increase in dystocia.

Table 3 Effect of pre-and post-weaning plane of nutrition on reproduc-
tion performance and colostrum production of ewe lambs (30-210 d of
age)

<u></u>	Post-weaning treatments					
Item	HQD		LQD			
	H-H	H-L	L-H	L-L		
Number of pregnant ewe (n=10)	10	5	7	2		
Pregnancy days (d)	149.1	147.8	146.6	-		
Lamb birth weight (kg)	2.7	2.8	3.6	-		
Lamb weaning weight at day 60 (kg)	15.2	14.8	16.5	-		
Lamb mortality	2 (10)	2 (5)	3 (7)	2 (2)		
Stillbirth	0 (10)	2 (5)	0(7)	2 (2)		
Colostrum (mL)	223	152	213	-		
Fat (%)	12.29	10.97	12.19	-		
Protein (%)	16.80	15.03	16.10	-		

HQD: high quality diet; LQD: low quality diet; 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).

Overfeeding animals causes internal fat deposits which obstruct the pelvic canal. All managers, however, must maintain a balance between achieving maximum frame growth without allowing excessive fat deposits. Fat animals will have high incidences of dystocia just as severely as underdeveloped animals (Wilson and Rossi, 2006).

The mortality rates in H-H, H-L, L-H and L-L sequences were 0%, 40%, 0% and 100% respectively (Table 3). High mortality of L-L and H-L groups may be related to their difficult parturition. Environmental factor such as season of birth is responsible for approximately 55% of dystocia (Anderson, 1992). Survival lambs were less in L-L and H-L groups and this situation may be due to poor mother ewes and low colostrum produced of these mothers (Table 3). Newborn lambs from H-L group with a lower birth weight tended to be weaker and therefore to have more trouble suckling adequate amounts of colostrum to provide sufficient levels of antibodies in their blood for initial immune protection, which is in accordance with report of Ahmad *et al.* (2000) in Pak-Karakul sheep.

In their study, they found that maternal body status is more effective than the parturition position on lamb survival, so that dystocia had little impact on H-H and L-H groups lamb survival.

The provision of an adequate amount of high quality maternal colostrum is essential for the health and survival of neonatal lambs. Quantity of colostrum and fat percentage in colostrum did not respond to pre-pubertal plane of nutrition (P>0.05), but colostrum protein percentages in H-H sequence showed tendency to rise (Table 3) which might be related to higher concentrations of serum total protein (the results have not reported) in ewe lambs fed H-H sequence. The quantity and quality of colostrum can be influenced by various factors including breed, lactation number, age, health status, nutrition, body condition score at parturition and genetics, as well as environmental factors (Hart *et al.* 2009).

# CONCLUSION

Based on the results of the current study, most skeletal growth in Kurdish lambs was taken place in pre-weaning period and strongly influenced by pre-pubertal plane of nutrition. The results also showed that adequate skeleton size in Kurdish ewes is needed in minimizing the lambs' mortality associated with parturition. However, further researches are needed to evaluate the effects of nutrition and mobility on dystocia and lambs' mortality in first parturition.

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