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Effect of Early Weaning on Biochemical Indicator in Serum of Xinjiang Local Sheep Breeds in China

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Abstract: In the present study, effects of early weaned time on biochemical indicators in serum of lambs of Xinjiang local sheep breeds in China were analized. The lamb of Chinese Merino sheep (Xinjiang type) (M), Kazak sheep (K) and Bayinbuluk sheep (B) were used for as experiment animals. The lambs were sorted as three groups (G1 and G2) are experiment groups, early weaning at 40 and 60 days, respectively fed with milk replacer. The control group suckled with milk of ewes (G3). Blood samples were collected at 90 days of the lambs. The indicators in serum measured include: ALB, SUN, CHOL, GLU, TG, LDL, HDL, AST, ALT, LDH, Cp, IgG, IgM, IgA. The results show: As for biochemical indicators in serum reflecting nutrient material metabolism, Chinese Merino sheep (Xinjiang type): experiment groups are higher than G3 at SUN level, significantly ($p<0.05$). As for GLU level, G1 is significantly lower than G3 ($p<0.05$). G1 is significantly lower than G3 at CHOL level ($p<0.05$). There are no significant differences at other parameters among groups. Kazak sheep: experiment groups are higher than G3 at SUN level, significantly ($p<0.05$). As for the GLU level, G2 is significantly higher than G3 ($p<0.05$). As for CHOL, LDL, DHL level, experiment groups are significantly higher than G3 ($p<0.05$). Bayinbuluk sheep: As for SUN level, experiment groups are significantly lower than G3 $p<0.05$). As for GLU level, experiment groups are significantly higher than G3 ($p<0.05$). CHOL, TG, LDL and HDL level of lambs in serum were not significant affected by early weaning. As for the change of enzyme activity in serum reflecting stress of lambs, Chinese Merino sheep (Xinjiang type): experiment groups are significantly higher than G3 at AST activity ($p<0.05$), G1 is significantly lower than G3 at ALT activity ($p<0.05$), G2 is significantly lower than G3 at LDH activity ($p<0.05$). Kazak sheep: As for AST activity, G1 is significantly lower than G3 ($p<0.05$). As for the ALT, LDH activity, there are no significant differences among groups. Bayinbuluk sheep: ALT, AST, LDH activity in serum of lambs were not affected by early weaning. As for the change of Immunoglobulin and Cp in serum, Chinese Merino sheep (Xinjiang type): G1 is singnificantly lower than G3 at IgG level ($p<0.05$). Kazak sheep: Experiment groups are significantly lower than G3 at IgM level ($p<0.05$). Bayinbuluk sheep: there are no significant differences at IgA, IgM, IgG and Cp level among groups. In conclusion, early weaning have not negative effect on indicators in serum of Bayinbuluke lamb. As for weaning age, it is better for China merino (Xinjiang type) and Kazakh lamb to wean at 60 days, there is no significant differences weaned at 40 and 60 days for Bayinbuluke lamb.

Key words: Lamb, Kazark sheep, Bayinbuluk sheep, early weaning, biochemical indicators, China

INTRODUCTION

As the sheep industry continues to develop. Early weaned to lambs become an inevitable trend, it is not only promote the development of gastrointestinal system effectively of lamb, exert the potential of fast growth in lamb but also helps to restore body condition of ewes, shorten the generation interval, raising reproductive performance of ewes (Shigang *et al*., 2009). In addition the use of early weaning techniques, improve the productivity of lamb which have a significant role to can reduce pressure on natural grassland stocking, reduce the cost of raising.

Xinjiang is one of China's five major pastoral areas is the largest number of sheep herds in the province. Sheep industry play a very important role for regional economic development, improved living standards of people. Chinese Merino sheep is the fine-wool sheep which bred successfully in early 50's. Chinese Merino sheep have higher fleece weight, higher yield, goodquality wool,

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genetic stability etc. Chinese Merino sheep (Xinjiang type) the average weight of adult rams were 93.00 kg, ewes were 45.96 kg; The average fleece weight of ewes were 5.49 kg, the average yield were 2.96 kg. The average fleece weight of stud rams were 12.23 kg, the average yield were 6.09 kg; the average fiber diameter were 60-64 NM (Xinjiang People's Publishing House, 1988). Kazak sheep is an old hair sheep breed in Xinjiang which is dual purpose mutton and fat. Kazak sheep has the characteristics of sturdy, good grazing performance and high yield etc. The average weaning weight of lambs were 32.26 kg, the female lambs were 31.55 kg; the average weight of rams were 60.34 kg, ewes were 44.9 kg; the dressing percentage of Castrate sheep were 47.6% (Xinjiang People's Publishing House, 1988).

Bayinbuluke sheep is a good local breed which is hair sheep of dual purpose mutton and fat. The place of origin are alpine pasture meadow in the 2500-2700 m above sea level. It has the characteristics of prematurity, crude feed tolerance, cold resistance and adapted high altitude etc. The average weight of rams were 69.5 kg, ewes were 43.2 kg; the dressing percentage of Bayinbuluk sheep were 43-46% (Xinjiang People's Publishing House, 1988).

Biochemical mark (blood parameters) is an important method to determine diet status in animals just like in humans. Blood is important to examine tissues and to obtain data about the organism's general metabolic status, physiological efficiency, health and diet. Data related to blood parameters may be good indicators for observing effects of different diet practices (Yurtman *et al*., 1997). At present, the study of blood biochemical indicator with early weaning concentrated in piglets and calves, few relevant reports about lambs in domestic and foreign.

In this experiment, base on used early weaning technology in lamb and comprehensively analyzed the serum biochemical indicators of early weaned lambs of three sheep breeds in Xinjiang, elaborated the differences between naturally breast-feeding lambs and early weaned lambs on body physiological function. Meanwhile, This will lay a solid theoretical basis for Xinjiang local sheep breeds to further study early weaning age and appropriate diet nutrition.

MATERIALS AND METHODS

Experimental animals and groups: About 36 of Chinese Merino (Xinjiang type) lambs, 36 of Kazak lambs and 30 of Bayinbuluke lambs which are approximately date of birth, birth weight difference was not significant, respectively were used for the test. They are randomly divided into three groups in all breeds, male and female in half. Group 1 weaning at 40 days, group 2 weaning at 60 days. Control group: the lambs were fed with milk from their ewes.

Feeding management: According to the U.S. NRC (1985) nutritional requirements of sheep, according to the nutritional needs of early weaning weight 10 kg and 250 g daily gain to formulated the ration (Table 1). Ration amount supplied for the lambs was kept as 300 g day^{-1}, increasing the feeding amount in accordance with feed intake, growth and development of lambs. Daily feed amount was supplied in three parts at 08:00 am, 14:00 pm and 19:00 pm. Fresh and clean water was always made available for the animals. The study lasted totally 60 days. About 10 days of the period were for exercise and the rest 50 days were used for essential feeding period. Pens daily cleaned, disinfected once per week.

Sample collection and processing

Blood collection time: About 10 mL of blood sample was taken from jugular vein of each lamb at the same time of 90 days old (9:00-10:00 am). Blood samples were inclined to a place until the serum precipitated, standing a night at 4°C then taken it out centrifugation 15 min with 3000 r min^{-1} after 1 h at room temperature. Serum was harvested and stored at -20°C until assayed. After the study to determine on Albumin (ALB), Serum Ureanitrogen (SUN), Cholesterol (CHOL), Glucose (GLU), Triglyceride (TG), Low Density Lipoprotein (LDL), High Density (HDL), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Lactate Dehydrogenasis (LDH), Ceruloplasmin (Cp), Immunoglobulin (IgG, IgM, IgA) values.

These samples were analyzed by Abbott Company originated AEROSET automatic biochemical analyzer. Kits from the (Zhongsheng Beikong Bio-technology and Science Inc).

Data processing and statistical analysis: All values were expressed as mean±SD. One-way ANOVA variance analysis was conducted to determine whether or not a variation exists between the application groups. As a result of this variance analysis, LSD multi-comparison test was used to determine different group means. The 0.05 probability level was selected to determine statistical significance. All statistical analyses were conducted via SPSS 13.0 statistical packer program.

Table1: Nutrition elements of ration

Composition	Concentrate
Dry matter (%)	84.63
Crude protein (%)	21.46
Digestible energy (MJ kg^{-1})	13.13
Ca (%)	0.69
P (%)	0.56

RESULTS AND DISCUSSION

The change of biochemical indicators in serum reflecting nutrient material metabolism: As shown in the Table 2 China merino sheep (Xinjiang type): Compared with control group, the increase of ALB level was observed after early weaned but no significantly ($p>0.05$); As for SUN, the experiment groups are significant higher than control group ($p<0.05$); As for GLU, G1 is significantly lower than control group ($p<0.05$). As for HDL, G1 and G2 are significantly lower than control group ($p<0.05$). As for the activity of ALT, both G1 and G2 are significantly lower than control group ($p<0.05$). Experiment groups are significantly lower than control group at AST ($p<0.05$). As for the activity of LDH, G2 is significantly lower than control group ($p<0.05$). Among experiment groups, G1 is significantly higher than G2 ($p<0.05$).

Kazak sheep: As for ALB level, G1 is significantly lower than G2 ($p<0.05$). Experiment groups are significantly higher than G3 at SUN level ($p<0.05$). G2 is significantly higher than G3 ($p<0.05$) at GLU level. As for CHOL, LDL and HDL, experiment groups are significantly lower than G3 ($p<0.05$). G1 is significantly lower than G3 at AST ($p<0.05$). The activities of TP, TG, ALT and LDH in serum of lambs were not affected by early weaned.

Bayinbuluke sheep: As for SUN level, experiment groups are significantly lower than G3 ($p<0.05$). Experiment groups are significantly higher than G3 at GLU level ($p<0.05$). There are no significant differences among groups on TP, ALB, CHOL, TG, LDL, HDL, AST, ALT and LDH levels.

Among breeds: Experiment groups of M and K are significantly higher than control group at SUN level ($p<0.05$). G1 and G2 of M are 4.87 and 4.17 mmol mL^{-1} higher than that of G3 at SUN level, significantly ($p<0.05$). G1 and G2 of K are 4.01 and 3.85 mmol mL^{-1} higher than that of G3 at SUN level, significantly ($p<0.05$). It was observed that, SUN level of M lambs was larger increase. Experiment groups of B lambs are largest increase at GLU level in three breeds, respectively were 1.8 and 1.1 mmol mL^{-1} higher than that of control group ($p<0.05$). As for CHOL level, 40 days old weaned lambs of M and K were 0.31 and 0.91 mmol mL^{-1} lower than that of control group ($p<0.05$), K lambs had a larger decrease.

Experiment groups of K lambs had a largest decrease at HDL level in three breeds, respectively were 0.31 and 0.29 mmol mL^{-1} lower than that of control group ($p<0.05$). As for AST activity, K lambs had a largest decrease in three breeds, respectively were 25.95 and 23.87 U L^{-1} lower than that of control group ($p<0.05$).

Table 2: Biochemical indicators reflecting nutrient material metabolism in serum

Indicator	Breed	N	Group 1	Group 2	Control group
TP (g L^{-1})	M	12	62.15±5.39	64.15±4.95	60.09±5.06
	K	12	60.378±5.31	62.29±5.73	59.76±5.56
	B	10	60.68±9.210	64.82±9.98	71.32±8.76
ALB (g L^{-1})	M	12	27.09±1.22	27.76±3.13	26.28±1.74
	K	12	25.55±1.63^{b}	27.03±0.94^{a}	26.32±0.95^{ab}
	B	10	28.69±2.85	28.57±3.07	27.57±4.10
SUN (mmol mL^{-1})	M	12	12.01±2.16^{a}	11.31±2.80^{a}	7.14±1.00^{b}
	K	12	11.70±0.61^{a}	11.54±1.39^{a}	7.69±1.05^{b}
	B	10	8.63±1.43^{b}	9.73±1.46^{b}	11.91±1.23^{a}
CHOL (mmol mL^{-1})	M	12	1.77±0.24^{b}	1.93±0.26^{ab}	2.09±0.30^{a}
	K	12	1.71±0.02^{b}	1.81±0.18^{b}	2.68±0.63^{a}
	B	10	1.15±0.19	1.26±0.24	1.14±0.31
GLU (mmol mL^{-1})	M	12	1.95±0.36^{b}	2.49±0.45^{a}	2.46±0.36^{a}
	K	12	2.12±0.31^{ab}	2.71±0.55^{a}	1.90±0.49^{b}
	B	10	4.43±0.22^{a}	3.73±0.27^{a}	2.63±0.35^{b}
TG (mmol mL^{-1})	M	12	0.38±0.08	0.33±0.08	0.39±0.07
	K	12	0.33±0.02	0.36±0.08	0.38±0.09
	B	10	0.46±0.01	0.46±0.01	0.45±0.03
LDL (mmol mL^{-1})	M	12	0.40±0.10	0.48±0.07	0.46±0.11
	K	12	0.36±0.05^{b}	0.37±0.07^{b}	0.74±0.31^{a}
	B	10	0.33±0.07	0.40±0.09	0.47±0.03
HDL (mmol mL^{-1})	M	12	0.43±0.07^{b}	0.47±0.06^{b}	0.57±0.12^{a}
	K	12	0.42±0.03^{b}	0.44±0.06^{b}	0.73±0.02^{a}
	B	10	0.68±0.09	0.73±0.02	0.73±0.20
ALT (U L^{-1})	M	12	9.33±3.50^{b}	11.00±2.57^{ab}	13.55±4.97^{a}
	K	12	11.25±3.68	10.78±3.60	12.40±5.27
	B	10	18.26±4.62	16.45±3.63	16.57±1.02
AST (U L^{-1})	M	12	93.25±14.41^{b}	98.70±11.20^{b}	110.50±12.83^{a}
	K	12	98.25±16.25^{a}	100.33±15.64^{ab}	124.20±24.76^{b}
	B	10	99.17±15.69	96.20±19.61	97.31±8.92
LDH (U L^{-1})	M	12	420.45±58.81^{a}	365.17±40.75^{b}	455.31±70.41^{a}
	K	12	378.25±8.06	409.75±67.44	390.40±50.74
	B	10	574.91±77.84	545.96±67.35	516.68±87.23

^{ab}Values without or with similar superscripts are not different ($p>0.05$)

Table 3: Concentration of IgA, IgM, IgG and Cp in serum of lambs

Indicator	Breed	Group 1	Group 2	Control group
IgA (g L^{-1})	M	1.65±0.18	1.67±0.38	1.48±0.28
	K	1.57±0.27	1.44±0.15	1.62±0.33
	B	1.40±0.28	1.58±0.12	1.61±0.34
IgM (g L^{-1})	M	2.04±0.23	2.11±0.47	1.99±0.23
	K	1.70±0.27^{b}	1.72±0.13^{b}	2.31±0.37^{a}
	B	1.96±0.13	2.16±0.32	2.34±0.22
IgG (g L^{-1})	M	4.28±0.26^{b}	4.63±0.17^{a}	4.60±0.36^{ab}
	K	4.52±0.19	4.36±0.24	4.65±0.13
	B	4.35±0.26	4.43±0.32	4.48±0.19
Cp (mg mL^{-1})	M	109.25±5.92	123.46±24.51	130.73±10.21
	K	97.59±13.34	101.14±18.10	109.47±31.21
	B	124.87±20.73	110.20±18.05	135.60±22.96

^{ab}Values without or with similar superscripts are not different ($p>0.05$)

The change of antibody and Cp level in serum of lambs: As shown in Table 3 China merino sheep (Xinjiang type) as for the concentrate of IgA and IgM, experiment groups are higher than control group ($p>0.05$). As for the concentration of IgG, G2 is significantly higher than G1 ($p<0.05$).

Kazak sheep: Experiment groups are significantly lower than G3 at IgM ($p<0.05$). There are no significant differences among groups at IgA, IgG and Cp level.

Bayinbuluke sheep: Immunoglobulin (IgA, IgM and IgG) and Cp levels in serum of lambs were not affected by early weaning.

Biochemical indicators in serum reflecting nutrient material metabolism: SUN and ALB in serum reflect body protein absorption and metabolism. Caldeira *et al.* (2007) reported, the best metabolic protein indicator of sheep is blood albumin and urea values. This increase occurred in ALB level is a result of high protein content of the experiment feed. This indicates that ration protein consumed was converted into body proteins effectively. Lower SUN level indicates amino acid balance is good, higher rate of protein synthesis in the body. SUN level increases in case of high protein consumption, renal malfunctions and gastrointestinal blooding (Tas *et al.*, 2009). BUN (all blood including serum) levels may be seen in case of insufficient protein consumption and severe acute and chronic liver-diseases occurred due to any reason. In this study, Experiment groups of M and B lambs are higher than control group at TP and ALB level ($p>0.05$).

About 60 days old weaned lambs of K is higher than G1 at ALB level ($p<0.05$). Wang *et al.* (2007) reported that there was no significant difference between different treatments in blood serum index of ALB at 47 days which indicated that it was feasible for lambs to wean earlier, even at day 7. This result of the study is so consistent with the report of Nussbaum *et al.* (2002) which showed an increasing trend in ALB level on 7-28 days calves.

In this experiment, SUN level of early weaned lambs on M and K were significantly higher than control group ($p<0.05$). Indicate that the rumen of early weaned lambs may not completely develop, so the protein absorption and utilization of ration is not high. To some extent, early weaning had a negative effect on the protein metabolism. The SUN level increase of weaned lambs on M was larger than K, indicated that M was affected largely by early weaned. Early weaned lambs of B was lower than control group at SUN level ($p<0.05$), indicated that early weaned lambs had a higher protein digestibility of dietary for B.

Relatively constant blood glucose concentration is an important condition to ensure the normal metabolism of cells, maintain the normal function of tissues and organs. If lower level of GLU it stands for insufficient energy or lower digestion rate in ration (Wang *et al.*, 2007). Body fat of animals is the main form of stored energy. As energy source, fat has an important role to physical activities of animals. The metabolic indicator for energy balance in sheep is glucose and non-essential free oil acids (Caldeira *et al.*, 2007). The increased fat acid level increases triglyceride, apo B and VLDL production in liver. When VLDL increases in the circulation, HDL and VLDL begin to interact via Cholesterol Ester Transfer Protein (CETP).

In this study, as for GLU level, G1 of M is lower than G3 ($p<0.05$), G2 of K is higher than G3 ($p<0.05$), experiment groups of B are higher than G3 ($p<0.05$). Research indicates that early weaned lambs of B had a better utilization of energy of ration among three breeds.

As for the indicators of reflecting lipid metabolism (CHOL, TG, LDL and HDL), CHOL level in serum of experiment groups decreased for M, while G1 of M is significantly lower than control group ($p<0.05$). Experiment groups of M are significantly lower than control group ($p<0.05$). As for CHOL, LDL and HDL levels, experiment groups of K are significantly lower than control group ($p<0.05$). Xiangmei *et al.* (1999) reported that serum CHOL decreased after weaning. There are no significant differences between experiment groups and control group at CHOL, TG, LDL and HDL levels for B lambs.

About 40 days old weaned lambs of M and K were 0.32 and 0.91 mmol mL^{-1} lower than control group at CHOL level which the CHOL level of K lambs had a bigger decrease. These results indicated that, early weaning have a significant effect on the indicator of lipid metabolism for M and K lambs and have a greater effect for K compare with M. Early weaning is no significant effect for B.

The change of enzyme activity reflecting stress in serum: Most serum enzymes come from different tissues of animal. Its activity level had a relation with metabolism and functional status of certain organs. Body's ability to adjust and adapt depend on the function of tissues and organs largely. The activity of blood ALT is increase due to cell degeneration in liver. When cell degeneration reach until mitochondria AST level in blood is increased. In cirrhosis diseased and malignant tumor mass formation, serum transaminases, mainly AST, increase 4-5 folds. Extremely high serum LDH activities occur in actue liver damages. However, moderate changes may occur in LDH activity in case of chronic liver diseases. Extremely increased LDH activity implies existence of a carcinoma spread to especially liver, however, the reason for this increase may be pernicious anemia (Aminlari *et al*., 1994; Wang, 1995). Bogin *et al*. (1997) reported that the GOT activity of the chicken heart increased significantly under long-term high temperature environment.

From the experimental results, early weaning reduced the activity of AST, ALT and LDH on M and K, of which experiment groups of M are lower than control group at AST ($p<0.05$), meanwhile AST activity of 40 days old weaned lambs decreased on K. As for the activity of AST, ALT and LDH, there are no significant different between experiment groups and control group. The decrease activity of ALT, AST and LDH in serum of weaned lambs, indicated that early weaning can increase the stress resistance of the body can have a positive effect on the liver. HDL and AST activity in serum of K weaned lambs had a bigger decrease in three breeds, indicated that Kazak sheep had a less weaning stress, strong anti-stress ability after early weaned. Xiangmei *et al*. (1999) reported that the activity of AST, CK and LDH in serum could be used as indicators of stress in piglets. There are no significantly differences at the activity of ALT, AST and LDH in serum among groups on 14 days after weaning.

Lamb serum antibodies and changes of ceruloplasmin: IgG is the highest concentration of Ig molecule in the blood. It play a dominant role on mechanism of antibody-mediated defense has the function of anti-bacterial, anti-virus and anti-toxins etc. As for the most animals, the concentrate of IgM is the second after IgG, it is the primary Ig molecule of humoral immunity on the early stages. At the same time, it is the most effective Ig molecule to fix complement and enhance the phagocytosis of phagocytes. IgA is the immunoglobulin which rich in carbohydrates. IgA also has the immunological activity of anti-bacterial, anti-viral, anti-toxin and secretory IgA play an important role on respiratory and digestive tract for the local mucosal immunity (Nian-Xing, 2005).

Cp can promote decomposition of histamine, serotonin etc can constitute non-specific disease resistance of body. As a protein of acute phase response, it may be increased in many pathological cases such as infection, trauma and tumors (Chunyong, 2006). It will decrease in cirrhosis, copper metabolism disorders etc.

As for M lamb there is an increasing trend for the concentrate of IgA and IgM in serum compared with the control group, G1 is higher than control group at IgG ($p<0.05$). The concentrate of Cp decreased for the experiment groups, no significant ($p>0.05$). As for K lamb, IgM concentration of experiment groups is lower than control group ($p<0.05$). There are no significant different between experiment groups and control group at IgA, IgG and Cp in serum. It can be observed that under the experimental conditions, the early weaned lambs of B fed with a reasonable diet, will not impact on immune function in lambs. Pie *et al*. (2004) report: early weaned piglets showed a significantly higher IgG concentration on day 49. It is known that weaning increases the production of inflammatory cytokines.

Analysis as the weaning time, weaned lambs gradually adapting to the solid feed can be better to digest and utilize it can promote lamb growth and increase body immunity. According to the decline of milking ability for ewes and the increase of growth and development needs for lambs. The obtained nutrients of breast-feeding can not meet the growing demand for lamb. Blecha *et al*. (1983) reported that compared with natural suckling piglets, piglets weaned at 2-3 weeks old showing a significant immune suppression and lower resistance to disease. While the piglets weaned at 5 weeks there was no significant difference.

CONCLUSION

SUN levels in serum of 40 and 60 days old weaned lambs significantly increased on China merino (Xinjiang type) while HDL level significantly decreased in experiment groups. As for Kazak sheep, early weaning also significantly increased SUN levels of 40 and 60 days old lambs while CHOL and LDL levels decreased, show that early weaning significant effected the metabolism of body protein, glucose and lipid for the two breeds. As for Bayinbuluke lambs, early weaning significantly decreased SUN levels meanwhile increased GLU level, indicated that early weaned lambs of B have been gradually adapting to the solid diet have a better utilization of nutrients, under the experimental conditions.

AST, ALT levels in serum of weaned lambs significantly decreased on M. AST level of 40 days old weaned lambs also significantly decreased on K. There is no significantly effect at ALT, AST and LDH levels for B lambs, indicating that weaning is not any impact on liver function for three breeds lambs. Meanwhile, weaning can improve the body anti-stress and relieve the damage degree of liver, heart and kidney organs.

About 40 days old weaned lamb of M is lower than control group at IgG level, significantly. Experiment groups of K are lower than control group at IgM level, early weaning significant influenced the humoral immune function of lambs in two breeds. As for B lambs, there is no significant effect at antibody level and Cp in serum. This may be related to the characteristics of cold resistance and adapted high altitude but also indicated that reasonable artificial feeding can raise the humoral immune function in weaned lambs.

In conclusion, early weaning have not negative effect on indicators in serum of Bayinbuluke lamb. Compared with other two breeds, it has better growth and development. As for weaning age it is better for China merino (Xinjiang type) and Kazakh lamb to wean at 60 days there is no significant differences weaned at 40 and 60 days for Bayinbuluke lamb.

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