



# Effects of Different Energy Levels on the Milk Performance and Blood Chemistry in Jersey Cows in Tibet

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## ABSTRACT

**Background:** Owing to the extreme cold and high elevation, demand for fat by people in the plateau is great than those on the plains. Butter was used to supply of fat to the herders in areas as remote as rural Tibet and as one of the traditional food, it occupied an important place in the diet. Therefore, there is a significance in improving butter production in Tibet.

**Methods:** To explore the effects of energy levels on milk performance, blood chemistry and milk hydrolysis amino acid in Jersey cows in Tibet. Forty late-lactation Jersey cows were randomly assigned into 4 groups equally according to the level of energy: E1 (11.50 MJ/kg); E2 (12.0 MJ/kg); E3 (12.5 MJ/kg) and E4 (13.0 MJ/kg).

**Result:** The results indicated milk yield, milk fat content, total solids content, lactose content and solids-not-fat content increased in response to higher levels of dietary energy, whereas the greater ( $P < 0.0001$ ) value of milk protein content was observed at the lower levels of dietary energy. The greater plasma GPT content was obtained in group E4 ( $P < 0.0001$ ), whereas, the greater plasma TG and GLU were observed in group E3 ( $P < 0.01$ ). Plasma TG and LDH of group E2 were greater than those of another treatments ( $P < 0.0001$ ) and the plasma UN of group E1 was significantly higher among treatments ( $P < 0.0001$ ). The greater values of most hydrolysis amino acid and total AA in milk were observed at the lower level of group E1.

**Key words:** Blood chemistry, Energy, Hydrolysis amino acid, Milk performance.

## INTRODUCTION

Tibetan plateau located in the southwest of China with an average altitude of over 4000 m (Tan *et al.*, 2021). The area is characterised by an extremely harsh environment, namely, severe cold, hypoxia (Chen *et al.*, 2021). Owing to the extreme cold and high elevation, demand for fat by people in the plateau is great than those on the plains (Li *et al.*, 2021). Butter was used to supply of fat to the herders in areas as remote as rural Tibet and as one of the traditional food, it occupied an important place in the diet. Because of higher content of milk fatty, most Jersey milk is used to produce butter in Tibet (Brown and Waldron, 2013).

However, because of the special geographical environment and the long period of negligible or zero plant growth each year (7-8 months), the winter dormant period is a harsh period for bovine (Ding *et al.*, 2007), these objective factors were one of the main reasons that led to the lower milk production and quality in Jersey cows. It is well documented that high dietary energy input are critical for effective lactation (Wang *et al.*, 2014). Zhou *et al.* (2015) reported that milking performance increased in response to the higher levels of dietary energy. Alstrup *et al.* (2015) also reported that fat supplemented ration increased yield of milk and milk fat. However, intaking excessive energy for lactating cows increased insulin resistance compared with cows consuming adequate amounts of energy (Leiva *et al.*, 2015). However, little is known about the effects of dietary energy on late lactation performance in Jersey cows. This current study was conducted to evaluate the milk performance, blood chemistry and hydrolysis amino acid in milk of Jersey cows fed different levels of dietary energy.

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## MATERIALS AND METHODS

This work was conducted in the Rikaze city of Tibet in November 2020. The experiments were conducted according to the animal care guidelines of the Animal Care Committee, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha city, Hunan Province, China (No. KYNEAAM-2006-0015).

A randomized complete block design was used in this study. Forty multiparous Jersey cows ( $225 \pm 35$  kg) in the late stage of lactation were blocked into 4 groups to give 4 blocks. Throughout the trial, cows were housed in a tie-stall

facility, fed twice daily (0500 and 1600 h) and had free access to clean water. The experiment lasted for 6 weeks, before starting the formal experiment, all Jersey cows were fed the same diets for 2-wk and then randomly assigned to 1 to 4 treatments with different levels of energy in diets for later 4-wk experimental phase. The four experimental diets were formulated to meet the nutrient requirements of lactating cows according to NRC (2001). Ingredients and Chemical composition of experimental diets were presented in Table 1.

The orts were collected and recorded once daily. The diets offered and left behind were recorded daily for calculating DMI. Weekly composites of the concentrates, forage and orts were obtained from daily samples of about 0.5 kg stored at -20°C until analysis. The DM and CP of concentrates, forage and orts were analyzed using the procedures of the Association of Official Analytical Chemists (AOAC, 2002). The NDF, ADF and CF content of above-mentioned samples were determined using a Fibretherm Fiber Analyzer (Gerhardt, Bonn, Germany) according to Van Soest *et al.* (1991) with addition of sodium sulphite and alpha-amylase in the NDF analysis.

Cows were milked twice daily and individual milk yield was recorded at each milking. Milk samples were collected at 2 consecutive (p.m. and a.m.) milking midway through the 5<sup>th</sup> week of the experimental phase for conventional analysis. Concentrations and yields of fat, protein, lactose, total solids (TS) and solids-not-fat (SNF) were computed as the weighted means from p.m. and a.m. milk yields on each test day.

Blood sample was collected on the last day of the formal experiment at 0500, 0700 and 1100 h, respectively. 10 mL of blood samples were collected every point-in-time from the coccygeal vein into Vacutainer tubes which including anticoagulation. After sampling, tubes were kept on ice and immediately transported to the laboratory for processing. Blood was centrifuged at 4000 × g for 10 min at 4°C and plasma was stored at -80°C until used for assay.

Milk samples of fat, protein, lactose, SNF and TS were analyzed by infrared methods (Foss North America, Eden Prairie, MN; Ag-Source, Verona, WI). The indexes of blood chemistry were analyzed by kits (Beijing Leadman Biochemical Co., Ltd, Beijing, China) using auto-biochemical analyzer (Beckman C × 4, Beckman Coulter, Inc. USA).

For the determination of hydrolysis amino acid in milk, 1ml milk sample were taken and mixed with 1ml concentrated hydrochloric acid into a ampulla bottle, sealed with alcohol blast burner and then put it into bake oven for 24h at 110°C. After 24h and cooling, opened the ampulla bottle and transferred hydrolysate to 25 ml volumetric flask, wishing the ampulla with deionized water three times and transferred it into volumetric flask to scale. The solution was filter by micropore filtering (0.25 μ m) before determination. 20 μ L was used to test the hydrolysis amino acid by a HITACHI L-8800 automatic amino acid analyzer (Japan).

Results of milk production, milk quality and blood parameters were statistically analyzed using ANOVA and the GLM procedure of the Duncan's multiple range tests

**Table 1:** Ingredients and chemical composition of experimental diets.

Ingredients of diet	Treatments			
	E1	E2	E3	E4
	(kg/d • cow)			
Barley Straw	2.4	2.4	2.4	2.4
Corn stover silage	12.0	12.0	12.0	12.0
Concentrates	5.5	5.5	5.5	5.5
Corn meal	46.92	46.92	46.92	46.92
Soybean meal	36.07	36.07	36.07	36.07
Wheat bran	9.17	9.17	9.17	9.17
Fatty powder (%)	0	1.69	1.72	1.75
CaHPO <sub>4</sub>	1.5	1.5	1.5	1.5
CaCO <sub>3</sub>	1.3	1.3	1.3	1.3
NaHCO <sub>3</sub>	0.6	0.6	0.6	0.6
NaCl	0.5	0.5	0.5	0.5
Premix <sup>1</sup>	4.0	4.0	4.0	4.0
<b>Chemical composition of concentrate (% of DM)</b>				
DM (%)	94.34	95.18	94.62	94.91
ADF (%)	11.38	11.42	15.53	15.63
NDF (%)	28.23	31.22	34.21	34.66
CF (%)	11.71	11.82	10.90	11.29
CP (%)	13.16	14.08	14.01	14.98
NEL(MJ/kg)	11.50	12.0	12.50	13.0

<sup>1</sup>Premix (/kg): 113.85 g MgSO<sub>4</sub>•H<sub>2</sub>O, 2.69 g FeSO<sub>4</sub>•7H<sub>2</sub>O, 2.55 g CuSO<sub>4</sub>•5H<sub>2</sub>O, 9.54 g MnSO<sub>4</sub>•H<sub>2</sub>O, 9.60 g ZnSO<sub>4</sub>•H<sub>2</sub>O, 30 mg Na<sub>2</sub>SeO<sub>3</sub>, 60 mg KI, 180 mg CoCl<sub>2</sub>•6H<sub>2</sub>O, 500,000 IU Vitamin A, 60 kIU Vitamin D, 2000 IU Vitamin E.

were used to compare differences among the three treatments. A P-value of less than 0.05 was taken to indicate statistical significance.

## RESULTS AND DISCUSSION

### Milking performance

The milk fat content in group E4 was 38.35% greater ( $P=0.0129$ ) than that of group E3. The milk protein content of E1 treatment was 10.69% and 5.07% greater ( $P<0.0001$ ) than that of E2 and E3 treatments (Table 2), Milk lactose of group E4 was significant greater than that of E1 and E3 treatments ( $P<0.05$ ) and the content of total solids and solids-not-fat of group E4 was the highest among the treatments and it was 16.22% and 12.67%, 7.55% and 3.41%, 16.22% and 12.67% greater ( $P<0.0001$ ) than those of group E1, E2 and E3 treatments, respectively.

### Hydrolysis amino acid composition

Concentration of Asp, Thr, Ser, Glu and Gly for group E1, were on average about 34.09%, 32.23%, 36.11%, 33.25% and 32.69% higher than that of E2 treatment ( $P<0.05$ ), besides, there were also significant difference ( $P<0.01$ ) in

concentration of Val, Ile, Leu, Tyr, Lys, His, Arg and Pro for group E1 treatment than that of E2 treatment. Concentration of total AA was on average about 35.01% and 23.01% higher ( $P<0.01$ ) for E1 treatment than that of E2 and E3 treatments, respectively (Table 3).

### Blood biochemistry indexes

The plasma GPT concentration of group E1 and E4 was greater ( $P<0.0001$ ) than that of group E2 and E3 (Table 4). For the plasma TC and LDH, the greatest value ( $P<0.0001$ ) was obtained from group E2 among treatments. The TG concentration in plasma for E2 treatment was significant lower ( $P<0.05$ ) than that of E3 treatment. The greatest value of plasma UN concentration was found in treatment E1 and it was 31.29%, 36.87% and 46.59% greater ( $P<0.0001$ ) than that of E2, E3 and E4 treatments, respectively. The plasma GLU concentration of E3 treatment was 8.84%, 9.84% and 12.97% greater than ( $P<0.01$ ) that of E1, E2 and E4 treatments, respectively.

### Milk performance

High dietary energy input played a critical role in effective lactation (Wang *et al.*, 2014). Zhou *et al.* (2015) have

**Table 2:** Effects of different energy levels on milk production and quality in Jersey cows.

Items	Groups				SEM	P
	E1	E2	E3	E4		
Milk production (kg)	2.41	2.43	2.42	2.46	0.02	0.4564
Milk fat content (%)	3.22 <sup>ab</sup>	3.17 <sup>ab</sup>	2.79 <sup>b</sup>	3.86 <sup>a</sup>	0.22	0.0129
Milk protein content (%)	3.52 <sup>a</sup>	3.18 <sup>c</sup>	3.35 <sup>b</sup>	3.45 <sup>ab</sup>	0.04	<0.0001
Lactose content (%)	4.28 <sup>b</sup>	4.61 <sup>a</sup>	3.81 <sup>c</sup>	4.64 <sup>a</sup>	0.06	<0.0001
Total solids content (%)	10.47 <sup>b</sup>	10.46 <sup>b</sup>	9.68 <sup>c</sup>	11.25 <sup>a</sup>	0.18	<0.0001
Solids-not-fat content (%)	7.84 <sup>b</sup>	7.91 <sup>b</sup>	7.26 <sup>c</sup>	8.18 <sup>a</sup>	0.07	<0.0001

<sup>a-c</sup>Means simulation fermentation profiles within a row for different soybean molasses adsorbents combined soybean molasses as fermentation substrates that do not have a common superscript differ ( $P<0.05$ ).

**Table 3:** Effects of difference energy levels on hydrolysis amino acid composition of milk in Jersey cows.

Items	Groups				SEM	P
	E1	E2	E3	E4		
Asp	4.68 <sup>a</sup>	3.49 <sup>b</sup>	3.73 <sup>ab</sup>	3.93 <sup>ab</sup>	0.32	0.0461
Thr	1.60 <sup>a</sup>	1.21 <sup>c</sup>	1.34 <sup>bc</sup>	1.41 <sup>b</sup>	0.06	<0.001
Ser	2.45 <sup>a</sup>	1.80 <sup>b</sup>	1.91 <sup>b</sup>	2.08 <sup>ab</sup>	0.14	<0.0001
Glu	11.34 <sup>a</sup>	8.51 <sup>b</sup>	8.98 <sup>b</sup>	9.76 <sup>ab</sup>	0.62	<0.01
Gly	0.69 <sup>a</sup>	0.52 <sup>c</sup>	0.55 <sup>bc</sup>	0.61 <sup>b</sup>	0.02	<0.0001
Val	2.48 <sup>a</sup>	1.91 <sup>c</sup>	2.08 <sup>bc</sup>	2.28 <sup>ab</sup>	0.10	<0.001
Ile	1.85 <sup>a</sup>	1.41 <sup>c</sup>	1.53 <sup>ab</sup>	1.69 <sup>bc</sup>	0.06	<0.01
Leu	4.10 <sup>a</sup>	2.94 <sup>b</sup>	3.35 <sup>ab</sup>	3.56 <sup>ab</sup>	0.19	<0.0001
Tyr	1.75 <sup>a</sup>	1.22 <sup>b</sup>	1.48 <sup>ab</sup>	1.43 <sup>ab</sup>	0.11	<0.01
Lys	2.45 <sup>a</sup>	1.94 <sup>b</sup>	2.09 <sup>b</sup>	2.30 <sup>a</sup>	0.07	<0.0001
His	0.87 <sup>a</sup>	0.67 <sup>c</sup>	0.74 <sup>b</sup>	0.80 <sup>b</sup>	0.02	<0.0001
Arg	1.01 <sup>a</sup>	0.77 <sup>c</sup>	0.88 <sup>b</sup>	0.90 <sup>b</sup>	0.03	<0.0001
Pro	1.43 <sup>a</sup>	1.13 <sup>b</sup>	1.26 <sup>b</sup>	1.52 <sup>a</sup>	0.06	<0.0001
Total AA	47.05 <sup>a</sup>	34.85 <sup>b</sup>	38.25 <sup>b</sup>	41.13 <sup>ab</sup>	2.52	0.0045

<sup>a-c</sup> Means simulation fermentation profiles within a row for different soybean molasses adsorbents combined soybean molasses as fermentation substrates that do not have a common superscript differ ( $P<0.05$ ).

**Table 4:** Effects of different energy levels on blood biochemistry indexes in Jersey cows.

Items <sup>1</sup>	Groups				SEM	P
	E1	E2	E3	E4		
GPT (U/L)	28.95 <sup>a</sup>	22.97 <sup>b</sup>	23.06 <sup>b</sup>	30.17 <sup>a</sup>	1.07	<0.0001
TC (mmol/L)	3.17 <sup>b</sup>	4.45 <sup>a</sup>	3.63 <sup>b</sup>	3.19 <sup>b</sup>	0.16	<0.0001
TG (mmol/L)	0.16 <sup>ab</sup>	0.14 <sup>b</sup>	0.22 <sup>a</sup>	0.17 <sup>ab</sup>	0.02	0.0133
LDH (U/L)	808 <sup>b</sup>	1001 <sup>a</sup>	860 <sup>b</sup>	818 <sup>b</sup>	24.11	<0.0001
UN (mmol/L)	5.16 <sup>a</sup>	3.93 <sup>b</sup>	3.77 <sup>b</sup>	3.52 <sup>b</sup>	0.18	<0.0001
GLU (mmol/L)	3.28 <sup>b</sup>	3.25 <sup>b</sup>	3.57 <sup>a</sup>	3.16 <sup>b</sup>	0.08	0.0038

<sup>1</sup> GPT = Glutamic-pyruvic transaminase; TC = Total cholesterol; GLU = Glucose; LDH = Lactate dehydrogenase; TG = Triglycerides; UN = Urea nitrogen.

<sup>a-c</sup> Means simulation fermentation profiles within a row for different soybean molasses adsorbents combined soybean molasses as fermentation substrates that do not have a common superscript differ (P<0.05).

concluded that milk yield, milk protein and yield and milk lactose yield increased in response to higher levels of dietary energy. The difference were ascribed to the different composition of diet and the species of cows probably, besides, the difference may be also result from the special geographical environment. Boerman *et al.* (2015) suggested that a high-forage diet that supplemented with palmitic acid increased milk fat yield, it was similar to the results in the current study. Zhou *et al.* (2015) reported that an increased energy level were beneficial to milk protein production due to increased yields of microbial protein when cows are fed corn stover as a dietary forage source. Cadorniga *et al.* (1993) also reported that high-energy diets stimulate microbial protein synthesis. The different dietary forage source and the plateau hypoxia and low temperature environment may be play a critical role in these difference and the partly energy maybe were used to maintain normal life activities of Jersey cows instead of MCP synthesis. Energy intake is associated with milk yield and milk solids production (Broderick, 2003). This agrees with the current study because the milk total solids content was greatest on group E4, the result in this study was also in accordance with the results reported by Reid *et al.* (2015).

#### Hydrolysis amino acid composition of milk

Many factors could affected the amino acid composition, which included dairy breed, parity and lactation stage and the content of amino acid in high milk protein was higher than that in low milk protein, the results in the current study was in accord with the reported by Zhou, 2017. However, few documents reported that how energy affected the content of amino acid in milk under the condition of the plateau hypoxia and low temperature environment and more further works were necessary to illuminate the mechanism.

#### Blood biochemistry

The activity of GPT could reflect the change of metabolism in body (Yan *et al.*, 2015). The result in the current study was in accordance with the results reported by Yan *et al.* (2015). TG and TC were important indexes that reflected body energy and fat metabolism and could reflect the fat

digestive and absorption directly (Yang *et al.*, 2013). Sun *et al.* (2013) suggested that the content of TG and TC in plasma increased with the increasing levels of dietary energy. The difference may have a relation to the lipid metabolism under the special geographical and climate environment in Tibet. There was a positive relationship between the concentration of milk UN and blood plasma urea nitrogen (Ciszuk and Gebregziabher, 1994). Jaster *et al.* (1990) reported that the high dietary energy for calf starters would result in the decrease of UN content in plasma, the result in the present study was in line with the results reported by Jaster *et al.* (1990).

Changes of plasma Glu content with the increasing level of dietary energy in the present study were multifarious. This phenomenon may be related to the plasma insulin content. Researchers have reported that dairy cows increased insulin resistance largely due to deficient energy intake for the postpartum period (Sinclair, 2010) and excessive energy intake for non-lactating dairy cows and lactating (Leiva *et al.*, 2015). Plasma LDH was an important enzyme that related to glycolysis and played a critical role in energy metabolism. The dietary energy level of E2 promoted gluconeogenesis in liver, which may be the main reason for the increase of plasma LDH content.

#### Conflict of interest

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