

Effects of Fasting on Body Weight, Serum Hormones, and Biochemical Parameters in Jiulong Yaks (Postprint)

Authors: Ren Jianlin, Wang Zhisheng, Quanhui Peng, Hu Rui, Zou Huawei, Jing Xiaoping, Pu Qijian, Zeng Shaoyu

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Abstract

The present study was designed to investigate changes in body weight, serum hormones, and biochemical parameters of Jiulong yaks during cold-season fasting stress. Eight healthy, disease-free, well-growing 2-year-old Jiulong yaks with a body weight of (90.75 ± 8.66) kg were randomly assigned to a control group and a fasting group based on similar body weight, with four replicates per group, one yak per replicate. The control group was fed normally, while the fasting group underwent feed withdrawal. The experimental period lasted 7 days. Body weight was measured and jugular vein blood samples were collected on days 1, 3, 5, and 7 of the experiment. The results showed that after 7 days of fasting, yak body weight decreased significantly ($P < 0.05$), serum glucose and insulin concentrations decreased significantly ($P < 0.05$), serum concentrations of non-esterified fatty acids, triglycerides, adiponectin, and glucagon increased significantly ($P < 0.05$), serum concentrations of the gluconeogenic precursors propionate and lactate decreased significantly ($P < 0.05$), while serum concentrations of glycerol and β -hydroxybutyrate, derived from body reserve mobilization, increased significantly ($P < 0.05$), and the activities of key gluconeogenic enzymes pyruvate carboxylase, fructose-1,6-bisphosphatase, phosphoenolpyruvate carboxykinase, and glucose-6-phosphatase in serum all increased significantly ($P < 0.05$). These results suggest that after 7 days of fasting, yaks began to extensively mobilize body reserves, upregulate gluconeogenesis, meet the normal metabolic demands of the organism, and maintain stable serum glucose levels for survival.

Full Text

Effects of Starvation on Body Weight, Serum Hormone and Biochemical Indexes of Jiulong Yaks

REN Jianlin, WANG Zhisheng*, PENG Quanhui, HU Rui, ZOU Huawei, JING Xiaoping, PU Qijian, ZENG Shaoyu

(Key Laboratory of Low Carbon Culture and Safety Production in Cattle in Sichuan, Animal Nutrition Institute, Sichuan Agricultural University, Ya' an 625014, China)

Abstract

This experiment was conducted to investigate the changes in body weight, serum hormone, and biochemical indexes of Jiulong yaks under starvation stress during the cold season. Eight healthy 2-year-old Jiulong yaks with good growth performance and consistent body weight [(90.75±\$8.66) kg] were selected and randomly divided into a control group and a starvation group according to the principle of similar body weight, with 4 replicates per group and 1 yak per replicate. The control group was fed normally, while the starvation group was deprived of feed. The experimental period lasted 7 days. Body weight measurement and jugular vein blood collection were performed on days 1, 3, 5, and 7 after the start of the experiment. The results showed that after 7 days of starvation, yak body weight decreased significantly ($P<0.05$), serum glucose and insulin contents decreased significantly ($P<0.05$), serum non-esterified fatty acid, triglyceride, adiponectin, and glucagon contents increased significantly ($P<0.05$), serum contents of propionic acid and lactic acid as gluconeogenesis precursors decreased significantly ($P<0.05$), while serum contents of glycerol and β -hydroxybutyric acid derived from body reserve degradation increased significantly ($P<0.05$), and the activities of key gluconeogenic enzymes including pyruvate carboxylase, fructose-1,6-bisphosphatase, phosphoenolpyruvate carboxykinase, and glucose-6-phosphatase all increased significantly ($P<0.05$). These results indicate that after 7 days of starvation, yaks begin to mobilize body reserves extensively, enhance gluconeogenesis metabolism to meet normal physiological requirements, and maintain stable serum glucose content to sustain life.

Keywords: starvation; yak; adiponectin; gluconeogenesis

Introduction

China has approximately 16 million yaks, accounting for over 90% of the world's total yak population. Yaks are mainly distributed in high-altitude regions such as Tibet, Qinghai, Sichuan, and Gansu, serving as essential living and production resources for herders on the Qinghai-Tibet Plateau and exerting important influences on local society, economy, and culture. The natural environment of the plateau where yaks live is extremely harsh, and current yak farming still adopts the traditional grazing and supplementary feeding system of "relying on

nature for animal husbandry,” generally practicing rotational grazing according to cold (winter-spring) and warm (summer-autumn) seasons. Statistics show that over 50% of grassland areas on the Qinghai-Tibet Plateau have forage production capacity below 375 kg per hectare, with a short grass growth period typically beginning in late April and withering by late September, resulting in a withered grass period lasting up to 7 months during the cold season. During this cold season withered grass period, yaks remain in a state of starvation for extended periods. Under extreme climate conditions with heavy snow and freezing temperatures, yaks may even face survival conditions of complete feed and water deprivation. After one cold season withered grass period, yaks can lose up to 30% of their body weight through fat loss, with mortality rates reaching approximately 15%. Investigations conducted between 2010 and 2012 in 60 regions across 5 major yak-farming provinces (Tibet, Qinghai, Sichuan, Gansu, and Yunnan) involving 410 herder households revealed that the key issue in inefficient yak farming is the “food and warmth” problem during the cold season, with starvation stress being the primary factor affecting yak survival, causing direct economic losses exceeding 10 billion yuan annually.

Starvation is a state of physiological imbalance caused by nutritional deficiency. Under the regulation of the body’s homeostatic mechanisms, glucose is the primary nutrient for maintaining physiological balance. Studies on starvation and acute hypoglycemia have shown that in the short term, the liver can maintain blood glucose homeostasis by enhancing glycogenolysis and gluconeogenesis. Gluconeogenesis is an important source of glucose in ruminants, but under cold season starvation conditions, exogenous nutrient intake is limited, reducing gluconeogenic precursors derived from food degradation. The body mobilizes body fat and protein for energy, and the massive degradation of fat produces large amounts of gluconeogenic precursors. However, there are currently no reports on the effects of cold season starvation on fat metabolism and gluconeogenesis in yaks and their molecular mechanisms. Adiponectin (APN) is one of the most abundantly expressed protein products in adipose tissue, capable of regulating energy balance and glucose homeostasis, and plays important roles in regulating fat metabolism, energy balance, and insulin sensitivity.

In recent years, cold season supplementary feeding of yaks has been a focus of yak nutrition research. Building on previous studies, this research used Jiulong yaks as experimental subjects to establish a starvation model and investigate changes in body weight, serum biochemical indexes, key enzymes regulating gluconeogenesis, hormones, and gluconeogenic precursors under starvation conditions, thereby accumulating fundamental data for understanding yak resistance to harsh environments.

1. Materials and Methods

1.1 Experimental Animals

Eight healthy 2-year-old Jiulong yaks with good growth performance and similar body weight [(90.75±\$8.66) kg] were selected as experimental subjects. The experiment was conducted at the experimental farm of Sichuan Agricultural University.

1.2 Experimental Design

Using a paired design, Jiulong yaks with similar body weight were paired and divided into a control group and a starvation group, with 4 replicates per group and 1 yak per replicate. The control group was fed normally, while the experimental group was deprived of feed. Based on Yang Feng' s research on animals' tolerance to hunger and referring to studies by Wei Xiaoqiang and Yang Bingzhuang et al., the starvation period for the experimental group was designed to be 7 days. All yaks had free access to water. The pre-experimental period lasted 21 days, and the formal experimental period lasted 7 days.

1.3 Basal Diet

The basal diet was formulated according to the nutritional requirements for beef cattle weighing 150 kg with a daily gain of 0.8 kg/d, as referenced in China' s "Feeding Standard of Beef Cattle" (NY/T 815-2004). The concentrate-to-forage ratio was 35:65. The composition and nutrient levels of the basal diet are shown in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis) %

Items	Content
Ingredients	
Corn	
Wheat bran	
Soybean meal	
Rapeseed meal	
Distilled grain	
Rice straw	
CaHPO ₄	
CaCO ₃	
NaHCO ₃	
NaCl	
Premix ¹	
Total	
Nutrient levels²	
DM	
NE _{mf} /(MJ/kg)	

Items	Content
CP	
NDF	
ADF	
Ca	
TP	

¹The premix provided the following per kg of the diet: VA 1,100,000 IU, VD3 44,000 IU, VE 3,300 IU, Cu 10 mg, Fe 50 mg, Mn 20 mg, Zn 30 mg, I 0.50 mg, Se 0.20 mg, Co 0.10 mg.

²NEmf was a calculated value, while the others were measured values.

1.4 Feeding Management

Before the experiment, all experimental yaks were uniformly numbered and subjected to immunization and deworming. Other disinfection and immunization procedures followed the standard farm management protocols. During the pre-experimental period, all yaks were housed in pens and fed twice daily (morning and evening).

1.5 Sample Collection and Measurements

Blood samples were collected via jugular vein indwelling needles before morning feeding on days 1, 3, 5, and 7. The collected blood was left to stand in a cool place for 30 minutes, then centrifuged to prepare serum, which was stored at -20 °C until analysis.

1.5.1 Body Weight Jiulong yaks were weighed on an empty stomach on days 1, 3, 5, and 7 of the experiment.

1.5.2 Serum Biochemical Indexes Serum contents of glucose (GLU), triglyceride (TG), non-esterified fatty acid (NEFA), albumin (ALB), total protein (TP), and cholesterol (CHO) were determined using an automatic biochemical analyzer (AU 5800).

1.5.3 Serum Hormone Indexes Serum insulin (INS) and glucagon (GN) contents were measured using enzyme-linked immunosorbent assay (ELISA) according to the kit instructions (purchased from Nanjing Jiancheng Bioengineering Institute).

1.5.4 Serum Gluconeogenesis Substance Contents Serum contents of propionic acid, lactic acid, glycerol, and β -hydroxybutyric acid were determined by gas chromatography. Using sulfosalicylic acid as a protein precipitant, serum was centrifuged at 6,000 r/min for 30 minutes, and the supernatant was used

to determine the content of the glucogenic amino acid alanine using a Japanese L-7 automatic amino acid analyzer.

1.5.5 Serum Gluconeogenesis Key Enzyme Activities The activities of glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, pyruvate carboxylase, and fructose-1,6-bisphosphatase in serum were measured using ELISA kits purchased from Nanjing Jiancheng Bioengineering Institute, with specific steps performed according to the kit instructions.

1.6 Statistical Analysis

Data were organized using Excel software. Body weight data were analyzed using paired-sample t-tests in SPSS 19.0, serum biochemical index data were analyzed using independent-sample t-tests for inter-group comparisons, and intra-group data were analyzed using one-way ANOVA.

2. Results

2.1 Effects of Starvation on Body Weight

As shown in Table 2, after 7 days of starvation, the final body weight of yaks in the starvation group decreased significantly compared with the initial body weight ($P < 0.05$), with a reduction of 12.50%.

Table 2 Effects of starvation on body weight of yaks (kg)

Items	Control	Starvation
IBW	89.50 \pm 8.89B	92.00 \pm 9.59A FBW 91.50 \pm 8.70A 80.50 \pm 8.27B

In the same column, values with different capital letter superscripts indicate significant difference ($P < 0.05$).

2.2 Effects of Starvation on Serum Biochemical Indexes

As shown in Table 3, serum glucose and cholesterol contents in the starvation group decreased significantly on day 3 ($P < 0.05$) and remained stable thereafter. Serum NEFA content increased significantly on day 3 ($P < 0.05$), decreased slightly on days 5 and 7, but remained significantly higher than on day 1 ($P < 0.05$). Serum total protein and albumin contents increased significantly on days 5 and 7, respectively ($P < 0.05$). Serum triglyceride content increased significantly on day 7 ($P < 0.05$). Compared with the control group, the starvation group showed significantly higher serum total protein (days 5, 7), albumin (day 7), triglyceride (day 7), and NEFA contents (days 3, 5, 7) ($P < 0.05$), and significantly lower serum glucose (days 3, 5, 7) and total cholesterol contents (days 3, 5, 7) ($P < 0.05$).

Table 3 Effects of starvation on serum biochemical indexes of yaks

Items	Groups	Day 1	Day 3	Day 5	Day 7	
GLU (mmol/L)	Control	6.55 \pm 0.63	7.05 \pm 0.76A	6.26 \pm 0.24A	7.00 \pm 0.51A	<i>Starvation</i> 6.47 \pm 0.25a 5.45 \pm 0.3

In the same row, values with different small letter superscripts indicate significant difference ($P < 0.05$). In the same column, values of the same index with different capital letter superscripts indicate significant difference ($P < 0.05$). The same applies below.

2.3 Effects of Starvation on Serum Hormone Indexes

As shown in Table 4, serum APN content gradually increased with prolonged starvation, rising significantly by 19.46% on day 7 ($P < 0.05$). Serum glucagon (GN) content increased significantly on day 3 ($P < 0.05$), reached its peak on day 5, and decreased slightly on day 7 but remained significantly higher than on day 1 ($P < 0.05$). Serum insulin (INS) content reached its lowest level on day 3, then gradually increased, but remained significantly lower than day 1 on days 3, 5, and 7 ($P < 0.05$). The insulin/glucagon ratio was significantly lower on days 3, 5, and 7 compared with day 1 ($P < 0.05$). Compared with the control group, the starvation group showed significantly higher serum APN (days 5, 7) and glucagon contents (days 3, 5, 7) ($P < 0.05$), and significantly lower serum insulin contents (days 3, 5, 7) and insulin/glucagon ratio (days 3, 5, 7) ($P < 0.05$).

Table 4 Effects of starvation on serum hormone indexes of yaks

Items	Groups	Day 1	Day 3	Day 5	Day 7	
APN (ng/mL)	Control	148.99 \pm 10.84	142.52 \pm 7.10	147.85 \pm 10.10B	147.89 \pm 9.68B	<i>Starvation</i> 149.73 \pm 5.

2.4 Effects of Starvation on Serum Gluconeogenesis Substance Contents

As shown in Table 5, serum contents of the gluconeogenic precursors propionic acid and lactic acid both decreased with prolonged starvation, being significantly lower on days 3, 5, and 7 compared with day 1 ($P < 0.05$). Serum glycerol and β -hydroxybutyric acid contents increased over time, being significantly higher on days 3, 5, and 7 compared with day 1 ($P < 0.05$). Serum alanine content increased significantly on day 5 ($P < 0.05$) but decreased on day 7 to a level not significantly different from day 1 ($P > 0.05$). Compared with the control group, the starvation group showed significantly lower serum propionic acid (days 3, 5, 7) and lactic acid contents (days 3, 5, 7) ($P < 0.05$), and significantly

higher serum glycerol (days 3, 5, 7), alanine (day 5), and β -hydroxybutyric acid contents (days 3, 5, 7) ($P < 0.05$).

Table 5 Effects of starvation on serum gluconeogenesis substance contents of yaks (mmol/L)

Items	Groups	Day 1	Day 3	Day 5	Day 7
Propionic acid	Control	2.29 \pm 0.13	2.33 \pm 0.08A	2.33 \pm 0.12A	2.37 \pm 0.11A
β -hydroxybutyric acid	Control	7.41 \pm 0.83	7.50 \pm 0.33A	7.88 \pm 0.12A	7.78 \pm 0.67A

2.5 Effects of Starvation on Serum Gluconeogenesis Key Enzyme Activities

As shown in Table 6, with prolonged starvation, the activities of key gluconeogenic enzymes in yak serum—including pyruvate carboxylase, fructose-1,6-bisphosphatase, phosphoenolpyruvate carboxykinase, and glucose-6-phosphatase—all increased, being significantly higher on days 3, 5, and 7 compared with day 1 ($P < 0.05$). Compared with the control group, serum activities of key gluconeogenic enzymes were significantly higher on days 3, 5, and 7 ($P < 0.05$).

Table 6 Effects of starvation on serum gluconeogenesis key enzyme activities of yaks (U/L)

Items	Groups	Day 1	Day 3	Day 5	Day 7
Pyruvate carboxylase	Control	92.16 \pm 3.88	93.09 \pm 4.37B	93.29 \pm 3.76B	89.28 \pm 2.32B

3. Discussion

3.1 Effects of Starvation on Yak Body Weight

Body weight loss is the most obvious phenomenon in animals under starvation conditions. Research has shown that when body weight loss exceeds 40% under starvation stress, the organism faces a survival crisis. Generally, weight loss in young animals under starvation is greater than in mature animals, possibly because young animals have higher metabolism to ensure growth and development. The rate of body weight loss also depends on two factors: first, different energy requirements due to variations in body weight, body temperature, and developmental status; and second, different allocation of nutrients within the body during starvation. Chaiyabutr et al. found that goats lost 16% of their

body weight after 2 days of starvation. Chwalibog et al. found that pigs lost 24% of their body weight after 4 days of starvation. This experiment showed that Jiulong yaks lost 12.50% of their body weight after 7 days of starvation, indicating that during starvation, yaks consume stored nutrients to provide energy for metabolism, resulting in body weight loss. Additionally, the rate of weight loss was slower than in other animals, suggesting that yaks living on plateaus have stronger resistance to harsh environmental conditions compared to pigs and goats.

3.2 Effects of Starvation on Yak Serum Biochemical Indexes

When animals face starvation stress, the body utilizes glycogen, fat, protein, and other nutrients to meet energy needs. Serum glucose and triglyceride contents directly reflect the body's energy supply status. In this experiment, yak serum glucose content decreased significantly on day 3 of starvation but slightly rebounded and remained stable at a relatively low level during the subsequent 4 days, similar to previous studies. Other research has found that serum glucose content decreases significantly in the early stage of starvation but changes less with prolonged starvation, maintaining a relatively constant level. This may occur because stable serum glucose content is a prerequisite for normal life activities, and reduced serum glucose under starvation conditions prompts the body to maintain stability through glycogenolysis and gluconeogenesis. Studies have shown that serum glucose and cholesterol can be maintained at relatively low levels during starvation, possibly through neuro-humoral regulation that reduces overall metabolic rate.

Serum triglyceride content is dynamically regulated through lipolysis and fat decomposition, with triglycerides produced by fat decomposition and degraded by triglyceride hydrolase into glycerol and fatty acids. NEFA, produced by neutral fat hydrolysis, can serve as an alternative energy source when glucose is depleted. This study found that yak serum triglyceride content increased significantly on day 7 of starvation, and serum NEFA content also increased significantly with prolonged starvation. From the perspective of serum glucose content stabilizing in the later starvation period, the body's energy supply gradually shifted from glucose to fat mobilization as starvation progressed.

Serum total protein consists of albumin and globulin, with albumin maintaining blood osmotic pressure and serving as a carrier for fatty acid transport. In this experiment, albumin and total protein contents remained unchanged in the early starvation period but increased significantly in the later period. Studies have found that serum albumin, globulin, and total protein contents in catfish decreased significantly after 21 days of starvation, differing from our results. The stable content in the early period may be because fat, as the second energy source, is preferentially metabolized, while in the later period, the body gradually utilizes tissue protein as an energy source, resulting in increased metabolic products (total protein and albumin).

3.3 Effects of Starvation on Yak Serum Hormone Indexes

APN is the only adipokine discovered to date that is negatively correlated with fat deposition. Liu et al. found that 24 hours of starvation increased APN gene expression in visceral fat but not in the longissimus dorsi muscle, consistent with Hu Ge' s research in mice. This study found that serum APN content in yaks increased with prolonged starvation, which could further influence glucose metabolism.

Insulin and glucagon are antagonistic hormones, with the former decreasing blood glucose and the latter increasing it. The increased serum insulin content in the later starvation period in this study may be due to elevated glucagon promoting insulin secretion, with both hormones working together to maintain serum glucose stability. The changing trends of insulin and glucagon were similar to those of glucose content, indicating that the body can regulate serum glucose stability through hormonal control during starvation. Both insulin content and the insulin/glucagon ratio decreased initially and increased in the later starvation period, finally maintaining a level lower than pre-starvation. De Bruijne et al. found that starvation significantly decreased serum insulin content, while Marliss et al. found in human fasting trials that serum glucagon increased then decreased, and insulin decreased then increased with prolonged fasting, consistent with our results.

3.4 Effects of Starvation on Serum Gluconeogenesis Substance Contents

Gluconeogenesis in ruminants occurs mainly in the liver and kidneys, with most glucose supplied by hepatic gluconeogenesis. Under normal conditions, propionic acid produced by rumen microbial fermentation is the main gluconeogenic precursor in ruminants, and glucogenic amino acids from protein degradation can also be deaminated to produce glucose for blood utilization. We speculate that weight loss in yaks under starvation is mainly due to lack of exogenous energy, causing the body to utilize its own fat and protein for energy, which simultaneously produces large amounts of glucogenic precursors that promote gluconeogenesis to maintain serum glucose stability.

Fatty acids and glycerol from fat decomposition can synthesize glucose through β -oxidation and reverse glycolysis pathways, and glucogenic amino acids from protein degradation can be converted to pyruvate for gluconeogenesis. Crocker et al. found that serum β -hydroxybutyric acid content increased significantly in walrus after starvation. Other studies have found that propionate can promote insulin secretion. In this study, serum contents of lactate and propionic acid from food decreased significantly after starvation, while contents of glucogenic precursors from fat and protein mobilization (glycerol, alanine, and β -hydroxybutyric acid) increased. This indicates that gluconeogenic precursors shifted from propionic acid under normal conditions to glycerol, glucogenic amino acids, and ketone bodies after starvation, ensuring continued gluconeogenesis.

genesis and stable serum glucose content.

3.5 Effects of Starvation on Serum Gluconeogenesis Key Enzyme Activities

Due to unique digestive physiology, gluconeogenesis is a distinctive feature of carbohydrate and energy metabolism in ruminants compared with monogastric animals. Besides gluconeogenic precursors, gluconeogenesis is also regulated by key gluconeogenic enzymes, hormones, and glucose transporters. Pyruvate carboxylase, fructose-1,6-bisphosphatase, phosphoenolpyruvate carboxykinase, and glucose-6-phosphatase are four key enzymes involved in gluconeogenesis regulation, and their expression levels and activities directly affect gluconeogenesis. Glucose-6-phosphatase and phosphoenolpyruvate carboxykinase are rate-limiting enzymes for hepatic gluconeogenesis, and their transcription levels determine gluconeogenesis speed. Vecchi et al. found that hepatic phosphoenolpyruvate carboxykinase gene expression increased in mice under starvation. This study found that activities of all four key gluconeogenic enzymes in yak serum increased significantly with prolonged starvation. Other studies have found that key glycolytic enzyme activities in blood decreased significantly with prolonged starvation, and research on Mongolian castrated sheep under starvation found that starvation significantly increased hepatic fructose-1,6-bisphosphatase activity. These results suggest that to maintain stable serum glucose content, the body can promote glucose synthesis by increasing gluconeogenic enzyme activities to maintain glucose homeostasis.

Conclusion

1. After starvation, yak body weight decreased, serum glucose content decreased significantly, while NEFA, glucagon, and APN contents increased significantly, indicating that yaks mobilize substantial body reserves to maintain metabolic needs.
2. After starvation, serum gluconeogenic precursors propionic acid and lactic acid decreased significantly, while products of fat and protein mobilization (glycerol and ketone bodies) increased significantly.
3. After starvation, serum gluconeogenic key enzyme activities increased significantly, demonstrating that yaks can maintain stable serum glucose content by enhancing gluconeogenesis under starvation conditions.

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