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Effect of Dietary Energy Source on Ruminal Fermentation, Venous Blood Gases and Various Blood Metabolites of Baluchi Sheep

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Authors' contributions

Author AM wrote the protocol performed the experiment and wrote the manuscript; author ARV supervised the experiment and revised both protocol and manuscript; authors ARV and MDM. performed the analyses of the study.

Original Research Article

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ABSTRACT

Aims: Present study was to determine the effects of dietary energy source diets such as glucogenic and lipogenic nutrients on ruminal fermentation characteristics, venous blood gases and concentration plasma metabolites in Baluchi sheep.
Study Design: Latin square design with 3 periods (28 days).
Place and Duration of Study: Department of animal science, Faculty of agriculture, Ferdowsi University of Mashhad, between March 2012 and May 2012.
Methodology: Experimental diets were a glucogenic, a lipogenic or a mixture of glucogenic and lipogenic diets (50:50). The animals were fed diets consisted of 50% chopped alfalfa hay and 50% concentrate. Ruminal fluid samples were collected before the feeding until 8 hour post feeding at day 24 of the each experimental period. Blood samples were taken from jugular vein before the feeding, 2, 4 and 6 h post feeding at day 26 of each period. In order to determine venous blood gases and pH, jugular blood samples were collected using heparinized syringes before and 4 h after feeding on day 27.
Results: Results indicated that glucose and insulin concentration was decreased in sheep consume lipogenic diet ($P<0.05$). There were not significant differences between diets in regard of both ruminal pH and ammonia nitrogen concentration. Furthermore, blood pH and venous blood gases did not differ among diets ($P>0.05$).
Conclusion: Feeding the mixture of glucogenic and lipogenic diets (50:50) improved

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plasma glucose and insulin concentration, while ruminal and blood pH were not altered. This phenomenon indicates the potent synergistic beneficial effect of mixed diet to supply energy source.

Keywords: Glucogenic; Lipogenic; Venous blood gases; Ruminal fermentation.

1. INTRODUCTION

In modern agriculture, genetic selection for intense production has resulted in animals that produce food for mankind at a rate beyond the capability of their inherent metabolic machinery [1]. This led to a situation with the dairy ruminant where they have difficulty in consuming enough energy to meet their requirements for maintenance and milk production, especially in early lactation. However, selection on high genetic merit for milk yield is only partially compensated by an increase in feed intake resulting in an ongoing increase in negative energy balance for dairy ruminant [2, 3]. A negative energy balance has been related with an increase in incidence and severity of metabolic disorders, like fatty liver, ketosis [4, 5, 6], an increase in incidence in infectious diseases [7] and ruminal acidosis [8]. Several nutritional strategies to reduce the incidence of metabolic disorders have been studied. Most studies aimed at improving the energy balance by increasing energy intake [9]. A common approach is increasing the energy density of the diet by e.g. dietary supplementation of energy dense ingredients like non-fiber carbohydrates [10] or by decreasing the forage to concentrate ratio [11]. The inclusion of fat in the diet is also a feeding strategy to increase net energy intake [12,13]. However, increasing the dietary energy density entails a risk of compromising dry matter intake. On the other hand, decreasing the lipogenic (L) to glucogenic (G) nutrient ratio has been suggested to improve the energy balance in early lactation [14]. Lipogenic nutrients in ruminants originate from fermentation of fiber to acetate and butyrate, dietary fat or are derived from body reserves. Glucogenic nutrients originate from starch that has escaped rumen degradation or gluconeogenesis. As a precursor of ruminal propionate, starch resource is a glucogenic precursor and is a commonplace therapy for energy deficit in milk cows [14]. Glucogenic feed may reduce the severity of ketosis and fatty liver, but increased incidence of (sub) clinical acidosis. Lipogenic nutrients decrease glucose and increase nonesterified fatty acid (NEFA) and *β*-hydroxybutyrate (BHBA) plasma levels. The current study was conducted to assess the effects dietary energy source diets on ruminal fermentation, venous blood gases and plasma metabolites of Baluchi sheep.

2. MATERIALS AND METHODS

2.1 Animals, Diets and Experimental Design

Three rumen fistulated Baluchi sheep were used as a 3×3 Latin square design with 3 periods (28 days). Each period included 21 days of adaptation and 7 days of sample collection. Diets consisted of 50% chopped alfalfa hay and 50% concentrate. Animals fed diets as 3% of body weight (900g DM day^{-1}). The ingredients of concentrates are presented in Table 1. Chemical composition of the diets, based on the realized total feed intake which measured as described in AOAC [15], is presented in Table 2.

Table 1. Ingredient (%) of glucogenic and lipogenic concentrates

	Concentrate	
Item (%)	**Lipogenic**	**Glucogenic**
Corn	---	23.8
Barely	---	20.2
Canola meal	6.7	13.8
Wheat bran	24	13.8
Soybean meal	10.2	27
Wheat pulp	29.2	---
Sunflower meal	20.4	---
Bergafat T-300[1]	8.1	---
$CaCO_3$	0.3	0.3
Mineral-vitamin premix[2]	0.5	0.5
Sodium bicarbonate	0.4	0.4
Salt	0.2	0.2
Total	100	100

[1]It contains fractionated palm fatty acids and the natural percentage of glycerol (Berg+Schmidt, Hamburg, Germany).[2]Composition of vitamin-mineral mix: Ca, 196.0 g/kg; P, 96.0 g/kg; Mg, 19.0 g/kg; Fe, 3.0 g/kg; Na, 71.0 g/kg; Cu, 0.3 g/kg; Mn, 2.0 g/kg; Zn, 3.0 g/kg; Co, 0.1 g/kg; I, 0.1 g/kg; Se, 0.01 g/kg and Vit A, 500000 IU/kg; Vit D, 100000 IU/kg; Vit E, 100 IU/kg.

Table 2. Chemical composition (% DM) of the glucogenic, lipogenic, and mixed diets[1]

		Diet[2]	
Factor	**Lipogenic**	**Mixed**	**Glucogenic**
DM	91	91	90
Crude protein	26.51	25.93	25.48
Neutral detergent fiber	38	31.2	30
Acid detergent fiber	17.5	14.1	10.7
Ether extract	11	6.7	3.5
Ash	6.2	6	5.7

[1]Based on realized feed intake.

[2]Chopped alfalfa hay: DM, 92 %; CP, 13.67 % DM; NDF, 48.3 % DM; ADF, 36 % DM; EE,1.2 %; Ash, 10.8 %.

2.2 Measurements and Sample Analysis

Ruminal fluid samples (10 mL) were collected on day 24 before the feeding and every 15 minutes post feeding until 8 h. Samples of ruminal contents were strained through four layers of cheese cloth and pH was measured using a portable pH meter (Metrohm 744, Switzerland). A volume of 10 ml of the filtrated ruminal fluid acidified with 10 mL of HCL 0.2 N and stored for later determination of ammonia nitrogen (NH_3-N) concentration. Ruminal NH_3-N was determined using distillation method (Kjeltec Auto Analyzer 1030 Tecator, tecator, Hoganas, Sweden). On day 26, blood samples were taken from jugular vein before the feeding, 2, 4 and 6 h post feeding with heparinized syringe. Plasma was obtained by centrifugation (15 min at 3500× g) and frozen at −20°C until analysis. Blood samples were analysed for glucose; while concentration of insulin just determined in pre feeding and 4 h post feeding samples. Analysis of mentioned blood metabolite was performed using commercially available kit on an auto-analyzer TARGA 3000, Italy (Glucose, Biosystem Ltd., Spain). Insulin concentration was determined using an RIA kit (Coat-a-Count Insulin, Diagnostic Products Corporation, Los Angeles, CA). In order to determine venous blood

gases and pH, jugular blood samples were collected using heparinized syringes before and 4 h after feeding on day 27. The syringes were chilled in an ice bath immediately and transported to the laboratory within 1 h. Blood partial pressure of gaseous O_2 dissolved in blood (pO_2), partial pressure of CO_2 (pCO_2), percent O_2 saturation and actual HCO_3^- concentration were measured by a pH/Blood Gas Analyzer (Stat Profile pHOx Plus blood analyzer, Nova Biomedical, USA).

2.3 Statistical Analysis

Data were applied to the mixed model of SAS [16] with the following statistical model of:

$Y_{ijklm}= \mu + A_i + B_j + C_k + D_l + (AD)_{il} + \varepsilon_{ijklm}$; where
Y_{ijklm} = dependent variable,
μ = overall mean,
A_i = treatment effect,
B_j = period effect,
C_k = effect of animal,
D_l = sampling time effect,
$(AD)_{il}$ = interaction effect of treatment and sampling time and
ε_{ijklm} = residual error.

Treatments were considered as a fixed effect and period and animal were considered as random effects [17]. The sampling time was included in the model as repeated measurement by using compound symmetry. Differences between least squares means were considered significant at ($p < 0.05$), using PDIFF in the LSMEANS statement.

3. RESULTS AND DISCUSSION

The ruminal pH and NH_3-N concentration data are shown in Table 3. Mean and minimum values of pH were not different among treatments. However, sheep received the glucogenic treatment had lower maximum value of ruminal pH relative to other treatments ($p= 0.04$). This observation was not confirmed by previous studies on replacing alfalfa silage with corn silage [18] or replacing a high-fat concentrate with a high-starch concentrate [19], where in both cases the ruminal pH decreased with increasing availability of glucogenic nutrients compared to lipogenic nutrients. Moreover, ruminal pH followed the same trend in all treatments (Fig. 1). Ruminal NH_3-N concentration was not influenced by the treatments (Fig. 2). In study of Ruppert et al. [18], ruminal NH_3-N concentration was not altered in dairy cows fed diets supplemented with different levels of tallow.

Table 3. Ruminal pH and ammonia nitrogen concentration (NH_3-N) in sheep receiving either glucogenic, lipogenic or mixed concentrate

Item	Treatments				
	Glucogenic	mixed	Lipogenic	S.E.M.[1]	P-value
pH					
Mean	6.28	6.17	6.13	0.13	0.50
Minimum	5.84	5.64	5.66	0.10	0.33
Maximum	7.04[a]	7.19[b]	7.28[b]	0.06	0.04
NH_3-N (mg dl^{-1})	19.34	21.51	22.96	2.76	0.40

[1]S.E.M.: standard error of mean.
[a–b]Means within a row with different superscripts differ (P<0.05).

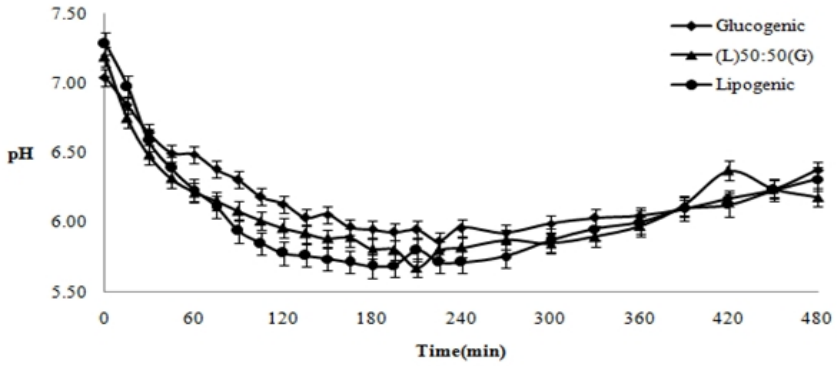

Fig. 1. Trend of rumianl pH in sheep fed glucogenic (G), lipogenic (L) or mix of both concentrate.

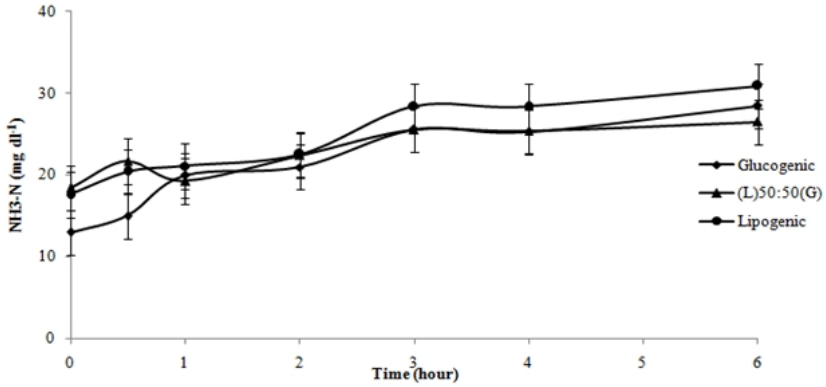

Fig. 2. Trend of ruminal NH_3-N concentration in sheep fed glucogenic (G), lipogenic (L) or mix of both concentrate.

Plasma glucose was greater ($P<0.05$) in sheep fed the mixture of both diets than other treatments. Furthermore, higher concentration of insulin was observed in mixed and glucogenic treatments compared to lipogenic treatment (Table 4). The increase of plasma glucose and insulin concentration in sheep consumed glucogenic diet is in agreement with results of van Knegsel et al. [14] who indicated that starch resource has an efficacious glucogenic effect that favors the increase of gluconeogenesis, glycogenolysis, or both. Increments in glucose and insulin concentrations were observed in all treatments 4h postfeeding (Fig. 3 and 4). Several studies presented a diurnal rhythm for glucose and insulin in ruminants [20, 21, 22]. Drackley et al. [9] observed greater glucose concentrations 4h postfeeding in cows fed high concentrate diet compared to the control. Moreover, Udum et al. [23] reported an elevation in plasma insulin concentration of lambs that were fed under different condition. This implies a time-after-feeding effect when interpreting dietary effects on blood metabolite and metabolic hormone.

Table 4. Blood metabolites in sheep receiving either glucogenic, lipogenic or mixed concentrate

	Treatments				
Factor	**Lipogenic**	**Mixed**	**Glucogenic**	**S.E.M.[1]**	**P-value**
Plasma concentration					
Glucose (mg dl^{-1})	70.83[a]	76.21[b]	72.64[a]	1.69	<0.01
Insulin (µIU mL^{-1})	53.30[a]	81.76[b]	80.92[b]	5.45	<0.01

[1]*SEM: standard error of mean.*

[a–b]*Means within a row with different superscripts differ (P<0.05).*

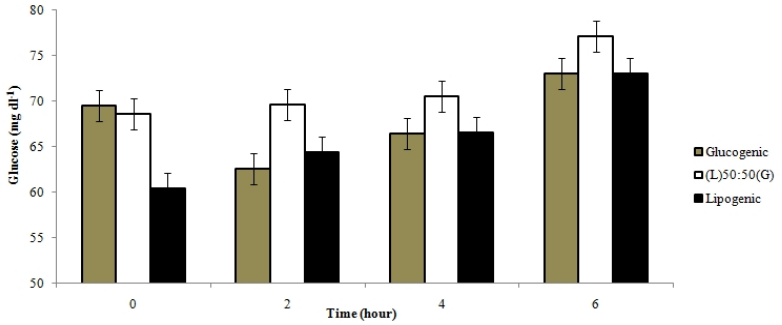

Fig. 3. Trend of plasma glucose concentration in sheep fed glucogenic (G), lipogenic (L) or mix of both concentrate.

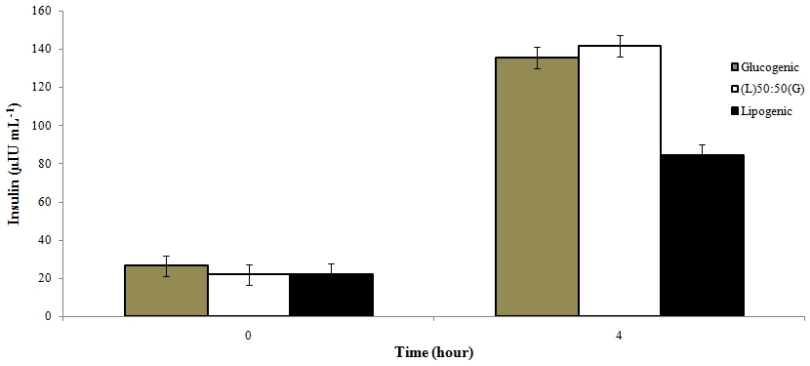

Fig. 4. Trend of plasma insulin concentration in sheep fed glucogenic (G), lipogenic (L) or mix of both concentrate.

Blood pH varies from 7.45 to 7.49 and did not differ among the animals fed the experimental diets (Table 5). Furthermore, diets had no impact on concentration of HCO_3^-, pCO_2, pO_2 and percentage of saturation oxygen (P> 0.05). Venous and arterial gas analysis is a clinical tool for determining acid-base status in animals [24]. Blood pH of healthy domestic animals is maintained in a very narrow range, between 7.35 and 7.45; values below 7.0 and above 7.7 are life-threatening [25,26]. The acid-base equilibrium of an organism is maintained by buffer systems; pulmonary, renal, and hepatic mechanisms; and bone activity [25,27]. Our data confirmed results of Sgorlon et al. [28] who indicated no difference between blood pH of ewes consumed high fat or control diet. However, high starch diet significantly diminished

blood pH. In addition, Apper-Bossard et al [29] conducted an experiment to assess the effect of different levels of concentrate on blood acid- base regulation in dairy cows. They illustrated that blood pH was not affected by feeding higher concentrate level.

Table 5. Venous blood gases in sheep receiving either glucogenic, lipogenic or mixed concentrate

		Treatments			
Item	**Lipogenic**	**mixed**	**Glucogenic**	**S.E.M.[1]**	**P-value**
Blood pH	7.450	7.483	7.497	0.03	0.45
pCO_2(mm Hg)	37.40	35.03	34.11	1.16	0.17
pO_2(mm Hg)	40.45	36.13	39.65	1.69	0.21
HCO_3^-(mEq lit^{-1})	25.81	25.76	25.94	1.90	0.99
O_2saturation (%)	73.68	67.78	75.45	3.89	0.38

[1]*SEM: standard error of mean.*

4. CONCLUSION

Under current study condition, feeding the mixture of glucogenic and lipogenic diets (50:50) improved plasma glucose and insulin concentration, while ruminal and blood pH were not altered. This phenomenon indicates the potent synergistic beneficial effect of mixed diet to supply energy source without undesirable effects on ruminal fermentation characteristics.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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