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EFFECTS OF DIFFERENT LEVELS OF FORAGE AND CONCENTRATE ON THE METABOLISM OF GLUCOSE, β-HYDROXYBUTYRATE AND NONESTERIFIED FATTY ACID IN LIVERS OF NONLACTATING GOATS

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ABSTRACT

The aim of this study was to investigate the effects of different levels of forage and concentrates diets on the metabolism of glucose, β-hydroxybutyrate (BOHB) and nonesterified fatty acid (NEFA) in livers of nonlactating goats. Six catheterized GuanZhong goats (40±2 kg weight) were fed high concentrate diet (HC, 40% hay, 60% concentrate) or high forage diets (HF, 60% hay, 40% concentrate). Blood samples were simultaneously taken from the portal, hepatic and arterial catheters at 0 h, before feeding, 2, 4, 6 and 8 h after feeding. The HC fed goats exhibited higher ($P<0.01$) plasma glucose concentration and arterial flow accompanied by NEFA, BOHB($P<0.01$) and hepatic glucagons concentrations ($P<0.10$) while NEFA portal flow and BOHB hepatic flow decreased ($P<0.05$). Glucose portal-arterial concentration difference and hepatic-portal concentration differences in BOHB and NEFA tended to decreased ($P<0.10$) with increasing NEFA portal and hepatic arterial concentration differences ($P<0.05$) in HC fed goats. There were no differences in net flow of glucose, BOHB and NEFA across splanchnic tissues, but a decreased net portal absorption in NEFA ($P<0.05$) accompanied with a tendency for depressing its net PDV plus hepatic uptake ($P<0.10$). In conclusion, the high concentrates diet may increase hepatic gluconeogenesis in nonlactating goats.

Key words: Concentrate, Liver, Glucose, Nonesterified fatty acid, β-Hydroxybutyrate, Goat

INTRODUCTION

Glucose is an important intermediary of metabolism in ruminants and nonruminants, as a major energy source in the pregnant animal and a primary precursor for lactose synthesis in lactation (Annison and Linzell, 1964). As glucose metabolism in ruminants differs from that of nonruminants because circulating glucose is mainly provided by gluconeogenesis in the liver, from precursors such as propionic acid or glucogenic amino acids. Glucose is also absorbed in smaller amounts by intestinal. The liver sits at the crossroads of metabolism and plays a key role in coordination of nutrient fluxes and all has the ability to sense the fuel needs all of the other tissues in the body and respond by adjusting its metabolism accordingly. It is well known that glucose metabolism has a close association with hepatic ketogenesis and lipid metabolism in liver where glucose metabolism is integrated with ketone bodies, nonesterified fatty acid (NEFA) and volatile fatty acids (VFAs) metabolism (Huntington *et al.*, 1988). In addition, the hormones, especially insulin and glucagon, play the major role of regulating intermediary metabolisms such that normal blood glucose concentration is maintained. A lot of previous researches have evaluated the impact of a dietary regime, a dietary composition or some materials relative to glucose production infusion on these metabolisms in pregnant or lactating ruminant animals (Storry and Rook, 1965; Huntington *et al.*, 1980; Kraft *et al.*, 2009; Kristensen *et al.*, 2010). But there were a few studies focused on the effect of different concentrate content in a diet on metabolism in nonpregnant or nonlactating ruminants. Our research is aimed to investigate the effect of varying in forage and concentrate on the metabolism of glucose, β-hydroxybutyrate (BOHB) and NEFA in liver of nonpregnant and nonlactating goats.

MATERIALS AND METHODS

Animal and Diets: Six catheterized nonlactating and nopregnant Guan Zhong dairy goats averaging 40±2 kg body weight (BW) were individually housed in metabolism stalls. These goats were assigned randomly to two groups of three goats each. Two diets were used in the experiment to meet or exceed slightly maintenance nutrients requirement of GuanZhonggoat, high concentrate diet (HC, 40% hay and 60% concentrate) and high forage diet (HF, 60% hay and 40% concentrate; Table 1), providing 0.33 and 0.30 net energy MJ/kg0.75, respectively. Goats were fed one of two diets in a randomized crossover design with two experimental periods. Each experimental period lasted 4 weeks, including first 2 weeks for adaptation. The diets were fed in equal portions (0.39 kg DM/goat) twice daily at 08:00 and 20:00. Water was available *ad libitum.*

Catheterization and Blood Collection: Surgical procedures and care of goats were approved by the Nanjing Agricultural University of Animal Care and Use Committee. Chronic indwelling catheters were installed surgically in the mesenteric, portal, and hepatic veins and a femoral artery to measure blood flow and net flow of across portal-drained viscera (PDV), liver and splanchnic tissues (liver+PDV). Goats were allowed to recover for at least 2 wk after surgery and to return to normal feed consumption before beginning the experiment.

From 08:00 to 16:00, six simultaneous blood sample sets were obtained at two hours intervals (0 h, before feeding, 2, 4, 6 and 8 hr after feeding) from the portal, hepatic and arterial catheters within each experimental period. The blood flow marker para-aminohippuric acid (PAH, 1% wt/vol sterile saline solution, Alfa Aesar, CAS 94-16-6, from Alfa Aesar China (Tianjin) Co., Ltd) infused continuously into the mesenteric vein at the rate of 0.8 ml/min using syring pump (SN-50F6, Sino Medical-Device Technology Co., Ltd., Shenzhen, China) following a priming rate (3 ml/min, for 5 min) given at 07:00. Samples were kept on ice and transported to the laboratory immediately, then were centrifuged at 1 469×g for 20 min at 4 ℃ to obtained plasma. The PAH was immediately determined and the remaining plasma was stored at -20 ℃ for further analyses.

Laboratory Analyses: Plasma flow rates were determined by using PAH, which were measured on plasma deproteinized by 0.5 mol/L trichloroacetic acid according to the procedures described by Katz and Bergman (1969). Plasma glucose and BOHB concentrations were determined using a commercial kit from KEHUA by auto biochemistry analyzer (Holland, WeiTu, selectra E). Plasma NEFA concentration was determined by using the test kit supplied JianCheng Bioengineering Institute (Nanjing, China). Plasma insulin and glucagon concentrations were determined by using ELISA kits from BluGene Biotech Company Limited (Shanghai, China).

Calculations: Portal and hepatic plasma flow rates were calculated from the plasma *p*-aminohippureate concentrations as described by Katz and Bergman (1969). The net portal release and net hepatic release of blood metabolites were calculated as described by Wieghart *et al*. (1986). General linear models (GLM) procedure of SPSS 16.0 was used for analysis of variance. This model included fixed effects of diet (D), period (P), sampling time (T) and interaction between diet and sampling time (D×T). The animal effect was assumed to be random in this model. Differences were declared significant at $P< 0.05$, and tendencies were declared for $0.05 < P< 0.10$ for all statistical tests.

RESULTS

Glucose: Glucose concentrations of hepatic and arterial veins ($P<0.01$, Table 2) and arterial flux (Table 3) were higher ($P<0.05$) for goats fed HC diet than that fed HF diet. Goats fed HC diet glucose hepatic flux ($P=0.06$, Table 3) and the portal-arterial concentration difference ($P=0.07$, Table 4) tended to higher than that fed HF. Net flux of glucose across splanchnic tissues is presented in Table 5. Negative net portal release values denoted a net utilization of glucose by PDV in both diets. Apparent glucose uptake by PDV was greater in HC vs. HF fed goats with -16.18 vs. -8.99 mmol/h respectively, reflecting an 80.02% increase. Positive net hepatic release and net PDV plus hepatic output denoted net production of glucose. Net hepatic release of glucose was more by 16.42% ($P=0.39$), while PDV plus hepatic output of glucose was less by 10.17% ($P=0.68$) in HC vs. HF group. There was no interactions between diet type and sampling time for concentration, flow and net flux across splanchnic tissues of glucose ($P>0.05$).

Table 1. Chemical composition and nutrient level of diets

Ingredient	High concentrate (HC)	High forage (HF)
Chinese wildrye	32.00	48.00
Alfalfa	8.00	12.00
Corn	43.17	28.78
Soybean meal	12.68	8.45
Limestone	1.25	0.77
Calcium phosphate dibasic	1.65	1.10
Salt	0.50	0.40
Premix[1]	0.75	0.50
Nutrient level		
Dry mater, %	88.60	88.90
Net energy, MJ/kg	5.89	5.40
CP[2], %	13.45	12.24
NDF[3], %	27.69	36.55
ADF[4], %	17.54	24.04

[1]premix provided: 3000, 1250, and 40 IU/kg of diet of vitamin A, D and E, and 6.25, 62.5, 62.5, 50, 0.25, 0.125, 0.125 mg/kg of diet of Cu, Fe, Zn, Mn, I, Se, Co, respectively; [2] CP = crude protein; [3] NDF = neutral detergent fiber; [4] ADF = acid detergent fiber.

β-Hydroxybutyrate (BOHB): BOHB portal, arterial and hepatic concentrations were lower by 17.65%, 20.41% ($P<0.05$) and 25.53% ($P<0.01$) in goats fed HC diet than that fed HF diet, respectively (Table 2). Compared with HF diet, HC diet decreased BOHB hepatic flow and hepatic-arterial concentration difference (Table 3 and 4, $P<0.05$), and tended to decreased hepatic-portal concentration difference ($P=0.06$, Table 4). There were no difference on net flux of BOHB across splanchnic tissues between two diets, but net hepatic release and PDV plus hepatic output were lower by 29.55 % ($P=0.16$)

and 23.59% (P=0.18) in goats fed HC vs. HF (Table 5). Except portal concentration and portal-arterial concentration difference, there was no interactions between diet type and sampling time for concentration, flow and net flux across splanchnic tissues of BOHB (P>0.05).

Nonesterified Fatty Acid (NEFA): The HC diet decreased NEFA concentrations of portal, hepatic veins and portal fluxes (P<0.05), significantly for arterial concentration (P<0.01, Table 2 and 3), while increased portal-arterial and hepatic-arterial concentration differences and net portal release (P<0.05, Table 4 and 5). NEFA hepatic flux and hepatic-portal concentration difference tended to decrease for goats fed HC diet compared with that fed HF diet (P<0.10, Table 3 and 4). Compared with HF diet, NEFA net PDV plus hepatic output tended to increase (P=0.07) while net hepatic release decreased by 28.64% (P=0.13) for goats fed HC diet (Table 5). There was no interaction between diet type and sampling time for concentration, flow and net flow across splanchnic tissues of NEFA (P>0.05).

Insulin and Glucagon: The plasma insulin and glucagon concentrations and flow of hepatic veins are given in the Table 6. The HC diet tended to enhance glucagon concentration of hepatic vein (P<0.10) as compared with HF diet. Insulin concentration of hepatic vein was greater by 10.04% (P=0.11) in goats fed HC vs. HF. There were no differences in insulin and glucagons flow of hepatic vein between two diets (P>0.05). There was no interactions between diet type and sampling time for hepatic concentrations and flow of insulin and glucagon (P>0.05).

Table 2. Effects of varying in forage and concentrate content on concentrations of glucose, β-hydroxybutyrate and nonesterified fatty acid in nonlactating goats

	HC	HF	SEM	P-Value		
				Diet	Time	DietòTime
Portal concentration ,mmol/L						
Glucose	3.651	3.459	0.083	0.115	0.019	0.829
β-Hydroxybutyrate	0.280	0.340	0.017	0.019	0.430	0.005
Nonesterified fatty acid	0.174	0.209	0.012	0.045	0.006	0.061
Hepatic concentration, mmol/L						
Glucose	4.174	3.845	0.060	0.001	0.002	0.588
β-Hydroxybutyrate	0.350	0.470	0.026	0.003	0.591	0.195
Nonesterified fatty acid	0.388	0.487	0.028	0.019	0.225	0.365
Arterial concentration, mmol/L						
Glucose	3.961	3.610	0.053	0.000	0.000	0.827
β-Hydroxybutyrate	0.195	0.245	0.013	0.011	0.007	0.362
Nonesterified fatty acid	0.537	0.690	0.035	0.005	0.385	0.389

Table 3. Effects of varying in forage and concentrate content on flow of glucose, β-hydroxybutyrate and nonesterified fatty acid in nonlactating goats

	HC	HF	SEM	P-Value		
				Diet	Time	DietòTime
Plasma flow, l/h						
Portal	57.619	59.165	2.284	0.379	0.014	0.175
Hepatic	93.875	92.177	2.562	0.945	0.001	0.608
Portal flow, mmol/h						
Glucose	213.770	205.854	10.998	0.615	0.011	0.288
β-Hydroxybutyrate	16.561	19.848	1.483	0.129	0.064	0.536
Nonesterified fatty acid	9.828	12.038	0.732	0.042	0.227	0.211
Hepatic flow, mmol/h						
Glucose	392.982	355.258	13.756	0.063	0.001	0.229
β-Hydroxybutyrate	33.940	42.413	2.845	0.045	0.157	0.712
Nonesterified fatty acid	36.390	44.640	3.057	0.067	0.296	0.711
Arterial flow, mmol/h						
Glucose	143.718	118.914	6.482	0.012	0.008	0.976
β-Hydroxybutyrate	7.390	8.386	0.681	0.311	0.006	0.495
Nonesterified fatty acid	19.848	22.954	1.715	0.212	0.163	0.553

Table 4. Effects of varying in forage and concentrate content on the venous-arterial concentration differences of glucose, β-hydroxybutyrate and nonesterified fatty acid in nonlacating goats

	HC	HF	SEM	P-Value		
				Diet	Time	DietòTime
Portal-Arterial concentration, mmol/L						
Glucose	-0.309	-0.150	0.060	0.071	0.843	0.216
β-Hydroxybutyrate	0.085	0.095	0.017	0.683	0.327	0.027
Nonesterified fatty acid	-0.363	-0.482	0.032	0.014	0.794	0.652
Hepatic-Portal concentration, mmol/L						
Glucose	0.523	0.386	0.063	0.136	0.803	0.515
β-Hydroxybutyrate	0.070	0.130	0.022	0.064	0.901	0.306
Nonesterified fatty acid	0.214	0.278	0.024	0.069	0.794	0.708
Hepatic-Arterial concentration, mmol/L						
Glucose	0.213	0.236	0.037	0.668	0.729	0.095
β-Hydroxybutyrate	0.155	0.225	0.024	0.045	0.675	0.309
Nonesterified fatty acid	-0.149	-0.204	0.015	0.016	0.175	0.044

Table 5. Effects of varying in forage and concentrate content on the net flow of glucose, β-hydroxybutyrate and nonesterified fatty acid across splanchnic tissues in nonlactating goats

	HC	HF	SEM	P-Value		
				Diet	Time	DietòTime
Net portal release, mmol/h						
Glucose	-16.177	-8.986	3.142	0.118	0.813	0.148
β-Hydroxybutyrate	5.053	5.508	1.183	0.788	0.595	0.190
Nonesterified fatty acid	-21.410	-28.253	2.274	0.043	0.524	0.990
Net hepatic release, mmol/h						
Glucose	35.495	30.490	4.020	0.387	0.906	0.144
β-Hydroxybutyrate	9.990	14.180	2.062	0.163	0.965	0.645
Nonesterified fatty acid	7.221	10.119	1.316	0.132	0.395	0.240
Net PDV plus hepatic output, mmol/h						
Glucose	19.318	21.504	3.671	0.677	0.928	0.136
β-Hydroxybutyrate	15.044	19.688	2.394	0.182	0.943	0.591
Nonesterified fatty acid	-14.696	-18.604	1.460	0.070	0.063	0.107

Table 6. Effects of varying in forage and concentrate content on the hepatic concentrations and flow of insulin and glucagon in nonlactating goats

	HC	HF	SEM	P-Value		
				Diet	Time	DietòTime
Hepatic concentration, ng/L						
Insulin	5.271	4.790	0.206	0.110	0.001	0.261
Glucagon	13.176	11.219	0.766	0.082	0.001	0.528
Hepatic flow, mg/h						
Insulin	0.479	0.449	0.024	0.384	0.000	0.887
Glucagon	1.171	1.043	0.078	0.255	0.005	0.356

DISCUSSION

Glucose is an important intermediary of metabolism in general and is particularly important for foetal growth and lactation. Foetus and uterus utilize glucose as a major energy source in the pregnant animal (Lindsay, 1973) and large quantities of glucose are removed by the mammary glands for lactose synthesis in lactation (Annison and Linzell, 1964). In ontrast to non-ruminants, little carbohydrate in ruminants is absorbed as glucose due to the ruminal fermentation. Thus, glucose needs must be hepatic production from precursors. In ruminants, the major glucogenic substrates are propionate, lactate/pyruvate, amino acids and glycerol

with the propionate being the major glucose precursors in fed animal (Lomax and Baird, 1983; Danfær *et al.*, 1995). In present study, apparent glucose uptake by PDV was greater in HC vs. HF (i.e, net portal release = -16.18 mmol/h vs. -8.99 mmol/h), and the glucose net released from liver were 35.50 mmol/h vs. 30.49 mmol/h, while there was no difference in net glucose PDV plus hepatic output between two diets. .The results suggest that the HC diet provide more glucose to utilize. It is presumed that the more propionate yield would be produced and removed to synthesize glucose by liver tissues for HC diet. Huntington and Reynolds, (1986) reported that abomasal infusions of glucose and starch could increase arterial plasma concentration and net absorption of glucose. Lemosquet *et al.* (2009) found that isoenergetic infusions (5.15 Mcal/d of digestible energy) of glucose into the duodenum (7.7 mol/d), propionic acid into the rumen (14.1 mol/d) increased whole body glucose rate of appearance by 48.41% and 19.52% in lactating Holstein cows, respectively. In our other experimental (unpublished data) results showed that a positive net portal uptake of propionate was observed in two diets and the HC diet increased net portal uptake of propionate as compared with HF diet (33.72 vs 26.07 mmol/h for HC vs HF diets, $P<0.05$); the net hepatic propionate releases showed a negative value in two diet and more propionate was utilized by liver tissues in HC diet (Net hepatic release =-29.14 vs -24.07 mmol/h for HC vs HF diets, $P<0.05$), which corresponded with the present study and confirmed presumption above. Annison *et al*. (1974) found that high concentrate diet increased molar proportion of propionic acid in the rumen and increased portal propionate concentration in adult cows. Sutton *et al.* (2003) reported that the molar proportions of propionic acid in rumen fluid was 18.9 and 37.0 mol/100ml for cows fed normal and low roughage diets, respectively, and net production of total VFAs was increased in cow fed low roughage diets.

In the present study, plasma concentration and flow of BOHB decrease ($P<0.05$) or tend to decrease ($P<0.10$) in HC diet compared with HR diet associated with the same change in NEFA, and though dietary concentrate content had no influence on net flow of NEFA across splanchnic tissues, HC diet decreased net portal absorption ($P<0.05$) and tended to decreased net gut plus hepatic output of NEFA ($P<0.01$), which fitted the observations obtained for plasma glucose concentration and net flow across splanchnic tissues. The primary ketone body released into portal blood is BOHB (Katz and Berqman, 1969). The anti-ketogenic effect of glucose is mainly to suppression of hepatic ketogenesis by two identified anti-ketogenic mechanisms, decreasing the supply of free fatty acids to liver and eliciting metabolic changes within the liver itself that are likely to lead to a decrease in the rate of hepatic ketone formation (Treacher *et al.*, 1976). Thus the decrease in the plasma concentrations and flow of NEFA in the present study was presumably due to increasing supply of glucose by gluconeogenesis from propionic acid in HC diet. This is possibility that fatty acid mobilization in peripheral adipose tissues might be depressed and the rate of triglyceride synthesis might be stimulated in HC diet, so that less free fatty acid release into blood to available for hepatic uptake and oxidation. Treacher *et al.* (1976) reported that glucose infusion via a jugular vein into the normal fed and unfed cows in early lactation for 48 h increased glucose plasma concentration associated with the decreased concentrations of BOHB, NEFA and glycerol ($P<0.05$). Furthermore, the changes in concentrations of these blood constituents occurring in the unfed cows were of much more magnitude than those occurring in the well-fed cows. The negative net portal release and net PDV plus hepatic output of NEFA associated with positive that two values in BOHB in the present study indicated NEFA was utilized and BOHB was produced by liver in two diets (Table 5). In addition, a decreased net splanchnic flux of NEFA and BOHB in HC diet confirmed the discussion above (Table 5).

Two pancreatic hormones, insulin and glucagon play important roles in maintaining normal blood glucose concentration by regulating intermediary metabolism in ruminants. The secretion of insulin and glucagon can be influenced by many regulators, like as glucose, certain amino acids, volatile fatty acids, adrenal medullary hormones. Our research found that the HC diet enhanced secretion of insulin and glucagons in nonlacting goats compared with HF diet (Table 6). This may be due to more propionic acid and glucose yield produced stimulated insulin and glucagons release. Several researches had found that infusion of propionate or glucose could stimulate insulin and glucagons release (Manns and Boda, 1967; Manns *et al.*, 1967; Bassett, 1971; Aiello *et al.*, 1984). Insulin and glucagon may in turn regulate ruminant metabolism. Insulin acts primarily on extrahepatic tissues as a storage and anabolic hormone, specifically adipose tissue and muscle. Its role is to enhance lipogenesis and protein synthesis by promoting glucose, acetate, free fatty acid and amino acids into peripheral tissues. On the other hand, glucagon is primarily involved in regulation at hepatic tissues. Its role is to increase hepatic glucose output and lipolysis by enhancing adipose tissue mobilization and hepatic uptake of certain glucose precursors and gluconeogenesis, so that it has a net effect of reducing that glucose precursors like as propionate, lactate/pyruvate, amino acids and glycerol available for nonhepatic tissues (Brockman, 1978). This presumption was proved by the observations of glucose, BOHB and NEFA in this present research. In addition, it was found that the time affected the majority of values on concentrations and flow but in which no diet× time interactions were observed in the present research (Table 2 and 3), suggesting diet and sampling time each

contributed to these values but not interactive. Many researchers found that glucose metabolism would change with time after feeding in sheep or dairy cow (Armentano *et al.,* 1984; Reynolds and Huntington, 1988; Fujita *et al.,* 2006). In view of the present and previous results, the response to feeding in glucose metabolism may be influenced by glucogenic precursors especially for propionate availability after feeding due to more rapid fermentation of starch than of fiber. Ross and Kitts (1973) observed that concentrations of propionate, acetate and butyrate were higher at 6 hr post feeding than at zero time in Dorset Horn weathers. These results are similar to results indicated by Evans *et al.* (1975) in Holstein heifers and mixed breed rams fed low-roughage or high-roughage. They found with both cows and sheep, concentrations of propionate, acetate and butyrate generally in creased after feeding so that the values obtained between 1.5 to 5.5 hr post-feeding were greater than 0.5 hr pre-feeding and 7.5 hr post-feeding values. In our other experimental results (unpublished data) also showed that the concentrations and net PDV plus hepatic outputs of propionate, acetate and butyrate increased with time after feeding and reached peak at 2-6 hr post feeding.

On the basis of present study on nonlactating goats it was concluded that HC diet would increase plasma glucose concentration and arterial flow accompanied by NEFA and BOHB concentrations and NEFA portal flow and BOHB hepatic flow decreased associated with a tendency for increased glucagons hepatic concentration compared with HF diet. In addition, HC diet enhanced NEFA portal-arterial and hepatic-arterial plasma concentrations differences and reduced BOHB hepatic-arterial concentration difference relative to HF diet. Furthermore, net portal absorption of NEFA decreased and net PDV plus hepatic absorption of NEFA was tended to decrease when feeding HC die to nonlactating goats. Diet and sampling time had no interaction.

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