Net nutrient flux in visceral tissues of goats fed diets based on maize or wheat[*]

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ABSTRACT

The objective of this study was to investigate the effects of dietary starch sources on the net portal-drained viscera (PDV) flux of metabolites in goats fed diets based on maize and wheat, respectively. Eight 8-month-old Liuyang Black wether goats, with catheters surgically in the mesenteric vein, the portal vein and the carotid artery, were assigned to one of two diets for determination of net nutrient flux across the PDV. There were no differences (P>0.05) in plasma concentrations of glucose and ammonia-nitrogen (N) in the portal vein and carotid artery, and net PDV flux of glucose and urea-N between maize diet and wheat diet. The portal plasma urea-N concentration and net PDV flux of ammonia-N of goats fed maize diet were lower (P<0.05) than those of goats fed wheat diet. Plasma concentrations in the portal vein and carotid artery and net PDV flux of certain essential amino acids of goats fed maize diet were higher (P<0.05) than those of goats fed wheat diet. The results of this study suggested that dietary starch source could affect N absorption and utilization in goats.

KEY WORDS: portal-drained viscera, starch sources, amino acid, urea-nitrogen, goat

INTRODUCTION

The degradability and digestibility of dietary starch in the rumen influence not only the extent of microbial fermentation and protein synthesis in the rumen, but also the availability of nutrients in the postruminal digestive tract (Taniguchi et al., 1995). Complicated interrelationships between starch and protein affected ruminal fermentation products (Ørskov, 1975), microbial synthesis (Hoover and Stokes, 1991), whole-body nitrogen (N) metabolism, and milk yield and composition in ruminants (Khan et al., 2007). Tagari et al. (2004) reported that portal-drained viscera (PDV) net fluxes of total free amino acid, glycine, isoleucine, leucine, phenylalanine, proline, serine, threonine, tryptophan and valine were greater in cows fed steam-flaked maize than that of cows fed steam-rolled maize.

Maize and wheat are grains commonly used as feeds for ruminants. The total starch contents of wheat and maize were 77 and 70%, respectively (Huntington, 1997). The starch from maize and wheat could be fermented in different manners in the rumen, the soluble starch (% total starch) of maize and wheat was 24 and 60%, and the potentially degradable starch (% total starch) of maize and wheat was 6 and 33% in the rumen, respectively (Offner et al., 2003). The soluble N (% CP) was 28 and 27%, and the potentially degradable N (% CP) was 65 and 71% for maize and wheat in the rumen, respectively (NRC, 2001). Obviously, the degradation characteristics of starch and N in the rumen differ for wheat and maize. The difference might result in variations in the ruminal microbial protein synthesis, which is mainly depended upon the fermentable energy and ruminal degradable protein (RDP), and availability of N and carbohydrate in the small intestine.

To examine this hypothesis, we have studied the rumen dynamic degradability of CP and starch of wheat and maize using in situ nylon bag technique, and found that the soluble N (% CP) and soluble starch (% total starch) were 34 and 74% for wheat, and 30 and 20% for maize in the rumen, respectively (Wang and Tan, 2007). On the basis of the above-mentioned study, the present study was conducted to investigate the effects of starch source (wheat vs maize) on net flux of essential AA (EAA) across the portal-drained viscera and plasma concentrations of glucose, ammonia-N and urea-N in the portal vein and carotid artery of goats.

MATERIAL AND METHODS

Animals and experimental design

The use of animals and the procedure of the experiment with animals were followed in accordance to the Animal Care and Guidelines of Institute of

Subtropical Agriculture, the Chinese Academy of Sciences.

Eight indigenous Liuyang Black male goats about 8 month-old with an initial liveweight (LW) of 20.0±0.5 kg were surgically fitted with permanent catheters in the mesenteric vein, portal vein and carotid artery. The surgical procedures were described by McLeod et al. (1997), and preparation of catheters and their maintenance during whole experiment were described by Huntington et al. (1989). The animals were held individually in stainless steel metabolic cages in a temperature-controlled (21ºC) and light-controlled (16 h light and 8 h dark) animal house. Fresh water was available during whole experimental period.

The goats were randomly divided into two groups, and assigned to two diets. The two diets consisted of maize stover and a mixed concentrate (Table 1). The amounts of the feeds offered to goats were controlled to provide nutrition at a plane of approximately 1.3 times of metabolizable energy requirement for maintenance (Lu and Xie, 1991). Both the concentrate and maize stover were fed separately, and the daily supply of both the diets were divided into equal portions and fed at 2 h intervals throughout the entire experimental period in order to keep the balanced supply of fermentable energy and RDP to satisfy microbial protein synthesis in the rumen.

Table 1. Ingredients and nutrient composition of experimental diets, % DM basis[1]

Item	Maize	Wheat
Ingredients, %		
maize stover	60	60
maize	25	-
wheat	-	25
rice bran	1	3
soyabean meal	10	11
fish meal	2	-
NaCl	1	1
Premix [2]	1	1
Nutrient composition		
ME, MJ/kg	10.2	9.9
crude protein	13.0	12.9
starch	14.9	15.0
ruminal degradable protein	5.40	5.72
neutral detergent fibre	40.2	40.5
Ca	0.65	0.68
P	0.28	0.29

[1] values, expressed on a DM basis, are the average of duplicates

[2] Premix contained per kg: g: $MgSO_4 \cdot H_2O$ 119, $FeSO_4 \cdot 7H_2O$ 2.5, $CuSO_4 \cdot 5H_2O$ 0.8, $MnSO_4 \cdot H_2O_3$, $ZnSO_4 \cdot H_2O$ 5: mg: Na_2SeO_3 10, KI 40, $CoCl_2 \cdot 6 H_2O$ 30; IU: vit. A 95.000, vit. D 17.500, vit. E 18.000

[3] metabolizable energy values were as reported by Zhang and Zhang (1998), and the others were determined values

Samples and sampling procedures

The experiment lasted for 12 days. Samples of feeds (the concentrate and maize stover) were taken daily, pooled, and sub-samples were taken. The feed refusals were collected daily, weighed, and pooled, and sub-samples were stored at 4°C for later analysis.

On day 12, a priming dose (15 ml) of p-aminohippuric acid (PAH, Serva GmbH, Heidelberg, Germany) solution (15 mg/ml) was given *via* the mesenteric vein at 07.30 h, followed by a constant infusion of PAH solution at a rate of 12 mg/min. Sixty min after the administration of the priming dose, 10 ml blood samples for each goat, from the portal vein and carotid artery were collected at 08.30, 11.30, 14.30 and 17.30 h. Sodium heparin solution (100 IU/ml) was used as anticoagulant. The samples were cooled in ice and transferred to laboratory. The blood samples were centrifuged at 3,000 *g* at 4°C for 20 min. The plasma was then divided into five aliquots and stored at -80°C for analyses of PAH, urea-N, ammonia-N, glucose and AA concentrations.

Chemical analysis

Milled feed samples were analysed for DM (AOAC, 1990), Kjeldahl N (AOAC, 1990) and starch (Zinn, 1988). The NDF was determined using the methods described by Van Soest et al. (1991), with heat stable amylase and sodium sulphite processing in NDF procedure, and results are expressed with residual ash.

Plasma glucose concentration was determined by the glucose oxidase (Nangjing Jiancheng Co., Ltd., Jiangsu, China) method using a chemical autoanalyzer (AutoAnalyzer II, Technicon Industrial Systems, Tarrytown, NY). Urea-N in plasma was assayed by the diacetyl monoxime method (Marsh et al., 1965) *via* automated analysis (AutoAnalyzer II, Technicon Industrial Systems, Tarrytown, NY). Ammonia-N in plasma was estimated using glutamate dehydrogenase method (Da Fonseca-Wollheim and Heinze, 1992). For AA concentration in plasma, the plasma sample was first deproteinized in a sulphosalicylic acid solution (60 g/l; containing 200 µmol/l nor-valine as an internal standard). Chromatographic separation of AA was performed on a Biotronik LC 5001 analyzer (Biotronik, Pusheim Bahnhof, Germany) according to the method of Moore and Stein (1954). Plasma PAH concentration was analysed according to the method of Eisemann et al. (1987).

Calculation and statistical analysis

Portal vein plasma flow (PVPF) was calculated by the equation (1) as follows:

$$PVPF = IR/(PAHpv - PAHa) \qquad (1)$$

where: IR - the infusion rate of PAH (mg/min), PAHpv and PAHa - PAH concentrations in plasma of the portal vein and carotid artery, respectively (mg/ml).

Net PDV flux of nutrient was calculated according to the difference between portal and arterial plasma concentrations multiplied by corresponding PVPF:

$$\text{PDV flux} = (Np - Na) \times PVPF \tag{2}$$

where: Np and Na - nutrient concentrations in the portal and arterial plasma, respectively.

There were four blood samples from each goat. The mean of four samples was calculated, and was used for the statistical analysis. To examine the difference between two diets, the t-test of two independent samples was performed. The P value of less than 0.05 was taken to declare statistical significance.

RESULTS

The average intakes (data not shown) of concentrate, maize stover, NDF, ADF, crude protein, starch and ruminal degradable protein (RDP) were 162, 242, 183, 115, 52, 60 and 22 g/d in maize group, and 164, 243, 184, 116, 52, 61 and 24 g/d in wheat group, respectively. The daily DM, NDF, ADF, CP and starch intakes of both groups were not affected by treatment (P > 0.05), but the RDP intake of goats in wheat group was higher (P<0.05) than that of goats in maize group.

Plasma concentrations of glucose, urea-N and ammonia-N are shown in Table 2. There were no differences (P>0.05) in glucose and ammonia-N concentrations in the portal vein and carotid artery, and net PDV fluxes of glucose and urea-N between the two diets. However, urea-N concentration in the portal vein and net PDV flux of ammonia-N for goats fed maize diet were lower than for goats fed wheat diet (P<0.05).

Table 2. Plasma metabolite concentrations of goats fed either a maize or wheat diet

Item	Maize	Wheat	SEM[1]	P [2]
Portal vein plasma flow, ml/min	662	670	10	NS
Glucose				
carotid artery mmol/l	3.59	3.47	0.06	NS
portal vein, mmol/l	3.41	3.32	0.26	NS
PDV flow, μmol/min	-123	-98.7	49.3	NS
Ammonia-N				
carotid artery, μmol/l	76.2	77.0	0.96	NS
portal vein, μmol/l	93.8	97.2	1.4	NS
PDV flow, μmol/min	11.7	13.5	0.52	*
Urea-N				
carotid artery, mmol/l	5.05	5.21	0.14	NS
portal vein, mmol/l	4.82	5.15	0.08	*
PDV flux, μmol/min	-124	-39.8	48	NS

[1] SEM - the pooled standard error of means; [2] NS - not significant; *, P<0.05

Essential AA concentrations in plasma are given in Table 3. The concentrations of lysine (Lys), methionine (Met), histidine (His) and EAA of goats fed maize diet in the carotid or portal vein were higher than those of goats fed wheat diet (P<0.05). The concentration of Thr in the carotid for goats fed maize diet was higher than for goats fed wheat diet (P<0.05).

Table 3. Plasma essential amino acids (EAA) concentrations in the carotid artery and portal vein of goats fed either a maize or wheat diet, μg/ml

Amino acids	Carotid artery		SEM[1]	P	Portal vein		SEM[1]	P [2]
	maize	wheat			maize	wheat		
Lys	16.4	13.1	0.56	**	19.3	15.3	0.50	**
Met	3.27	1.92	0.11	***	3.61	2.13	0.12	***
Thr	16.6	15.0	0.42	*	18.1	16.6	0.46	NS
Arg	13.9	14.0	0.56	NS	16.1	15.9	0.51	NS
His	3.57	2.14	0.08	***	5.78	4.42	0.13	***
Leu	16.1	15.9	0.55	NS	18.5	17.6	0.60	NS
Ile	9.04	8.60	0.35	NS	10.7	10.3	0.32	NS
Val	25.0	24.6	0.58	NS	28.5	27.5	0.57	NS
Phe	3.67	3.79	0.18	NS	4.53	4.62	0.18	NS
EAA	107	98.9	2.44	*	128	115	2.12	*

[1] SEM - the pooled standard error of means; [2] NS - not significant; * P<0.05; ** P<0.01; ***P<0.001

Net PDV flux of plasma EAA is listed in Table 4. Net PDV fluxes of Lys, Met and Val of goats fed maize diet were higher than those of goats fed wheat diet (P<0.05).

Table 4. Plasma EAA PDV net flux of goats fed either a maize or wheat diet, mg/min

Amino acid	Diets		SEM[1]	P [2]
	maize	wheat		
Lys	1.96	1.48	0.10	*
Met	0.23	0.15	0.03	**
Thr	1.02	1.13	0.14	NS
Arg	1.42	1.31	0.06	NS
His	1.46	1.53	0.07	NS
Leu	1.61	1.22	0.28	NS
Ile	1.12	1.16	0.06	NS
Val	2.25	1.94	0.14	*
Phe	0.57	0.56	0.03	NS
EAA	11.6	10.5	0.39	NS

[1] SEM - the pooled standard error of means; [2] NS - not significant; * P<0.05; ** P<0.01

DISCUSSION

Glucose. In the present study, no differences in plasma glucose concentration and net PDV flux of glucose between maize and wheat diets indicated that different starch sources (at least from maize and wheat) had no effects on glucose

net PDV absorption and metabolism. Our results were consistent with previous reports. Across a wide range of experiments encompassing varied diets, intakes and physiological states, net glucose absorption was almost always not changed, valued at zero or negative (Reynolds, 2006). The site of starch being digested had an impact on glucose supply at small intestine, however, it appeared that increases in glucose supply at the small intestine were accompanied by increased utilization of arterial glucose in tissues drained by the portal vein, such as mesenteric and omental fat depots (Reynolds, 2006). Circulating glucose in ruminants was mostly derived from gluconeogenesis which depended on glucogenic precursors mainly including propionate and glucogenic AA (Yong, 1977).

Ammonia-N.   In the present study, the net PDV ammonia-N flux of goats in wheat diet was higher than that of goats in maize diet. Nozière et al. (2005) reported that portal net appearance of ammonia tended to decrease from 108 to 73 mmol/h when steers were fed the diets based on wet and ground maize, and the diets based on dry and cracked maize, respectively. The results of present study and previous reports indicated that net PDV flux of ammonia-N might be affected by starch source and the processing of grain. Net PDV flux of ammonia-N was positively related to the ruminal ammonia-N concentration (Nozière et al., 2005). Therefore, the difference in net PDV flux of ammonia-N in goats between maize and wheat diets suggested there was difference in the ruminal ammonia-N concentration in goats fed maize and wheat diets. Ammonia-N concentration in the rumen can vary greatly depending on diet, time of feeding and feeding frequency, animal and other factors. Hristov et al. (2005) pointed out that ammonia-N concentration in the rumen was related to the release rate of N and carbohydrate, and ruminal fluid passage. According to our previous study (Wang and Tan, 2007), we found that the difference in soluble starch in the rumen between maize and wheat diets was much higher than the difference in soluble N for goats. On the other hand, daily RDP intake of per goat in maize group was lower than that in wheat group; furthermore, feeds were equally given every 2 h in the current study. Under this feeding regime, the difference in net PDV ammonia-N flux between maize and wheat diets might be probably ascribed to the differences in the release rate of starch and intake of RDP.

Urea-N. Plasma urea-N concentration is a useful index for the evaluation of N utilization by animal body (Sun et al., 2007). The results of present study suggested that dietary starch source (at least from maize or wheat) could affect N utilization by goats. It has been approved that plasma urea-N concentration of ruminant could be affected by dietary carbohydrate source (Osborne et al., 2002). The reasons for the current result might be related to higher net PDV flux of ammonia-N of goats fed wheat diet, which was turned into urea-N in the liver. In addition, certain higher plasma EAA concentration or EAA PDV net flux (such as Lys, Met, Thr and His) of goats fed maize diet might be due to other reasons.

It would be needed to do more exact experiments so as to obtain the reasonable explanation.

Amino acids. The current results indicated that the total amount of absorbed EAA from gastrointestinal tract (GIT) between wheat and maize diets did not differ, but the net fluxes of Lys, Met and Val differed. The quantity of net PDV flux of EAA reflected the quantity of absorbable EAA from the GIT. The microbial protein entering the duodenum is a dominant factor of determining EAA flow at the duodenum of dairy cows (Boguhn et al., 2006; Lapierre et al., 2006). Therefore, the reason for the difference in absorbed Lys, Met and Val from GIT between maize and wheat diets might mainly reflect the difference in the microbial protein entering the duodenum. Fermentable energy and RDP are essential for microbial protein synthesis in the rumen. Energy availability, or lack of synchrony between fermentable energy and RDP, could limit the utilization of available N by the ruminal microorganisms (Hristov et al., 2005). In the present study, the difference in RDP between maize and wheat diets was far less than the difference in starch degradation in the rumen, the release of carbohydrate was therefore likely to be more synchronous to RDP in maize diet compared with wheat diet, and the difference in synchronization of fermentable carbohydrate and RDP between wheat and maize diets might further result in the difference in the yield of ruminal microbial protein and influence the net PDV flux of AA. Tagari et al. (2004) also reported the difference in starch degradation rate of diet in the rumen of cows caused by different processing technique for maize had significant effects on the net PDV flux of free and peptide bound AAs, especially EAA of free AAs.

CONCLUSIONS

This study demonstrated that carbohydrate of maize was associated with higher, when compared with wheat, net fluxes of lysine, methionine and valine across portal-drained viscera (PDV) in goats, and associated with lower urea-N concentration in the portal vein and net PDV flux of ammonia-N. The results suggested that synchronization between fermentable carbohydrate and in the rumen ruminal degradable protein was advantageous to microbial protein synthesis and utilization of dietary nitrogen by goats.

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