

# Effects of different dietary ratio of physically effective neutral detergent fiber and metabolizable glucose on rumen fermentation, blood metabolites and growth performance of 8 to 10-month-old heifers

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**Objective:** The present study was undertaken to determine an optimal balance between the amount of physically effective neutral detergent fiber (peNDF) to metabolizable glucose (MG) on rumen fermentation, blood metabolites and growth performance of 8 to 10-month-old heifers.

**Methods:** A total of 15 healthy Holstein heifers weighing an average of 256 kg (8 month of age) were randomly assigned to three groups of five. Treatment diets consisted of the following three peNDF<sub>8,0</sub>/MG levels: 1.46 (Treatment A), 1.74 (Treatment B), and 2.08 (Treatment C).

**Results:** The results showed that the ratio of peNDF<sub>8,0</sub>/MG affected rumen fermentation, blood metabolites and growth performance of heifers. The average daily gain of heifers tended to decrease as the ratio of peNDF<sub>8,0</sub>/MG increased ( $p = 0.07$ ). The concentrations of blood urea nitrogen, triglyceride, and cholesterol increased significantly ( $p < 0.05$ ), while the high-density lipoprotein concentration decreased ( $p < 0.05$ ). After feeding 2 h and 4 h, insulin concentration in Treatment A was greater than Treatment C ( $p < 0.05$ ). Propionate concentration had decreasing trend ( $p = 0.07$ ); acetate to propionate ratio and non-glucogenic to glucogenic volatile fatty acid (NGR) increased significantly ( $p < 0.05$ ). In addition, the digestibility of dry matter, crude protein, neutral detergent fiber, and acid detergent fiber decreased significantly ( $p < 0.05$ ).

**Conclusion:** The present investigation indicated that dietary peNDF<sub>8,0</sub>/MG ratio can affect the growth and development, blood metabolites, rumen fermentation and apparent digestibility of heifers, and the optimal dietary peNDF<sub>8,0</sub>/MG ratio for 8 to 10-month-old heifers in the present study was 1.46.

**Keywords:** Physically Effective Neutral Detergent Fiber; Metabolizable Glucose; Rumen Fermentation; Blood Metabolites; Growth Performance

## INTRODUCTION

The composition of carbohydrates in diets is one of the key factors affect the growth and development of replacement heifers. It would lead to various metabolic disorders, if the carbohydrate composition in diet were imbalanced or not attaining nutrient requirement [1]. Traditionally, high-forage diets are often used in heifer herd feeding, but they are inefficiently digested due to high levels of lower digestibility forage [2]. Inefficient utilization of feeds by dairy heifers may reduce profitability. To minimize expenses during heifers rearing, high concentrate diets composed of high energy and high protein are being fed frequently to accelerate growth rates of calves and heifers. It has been demonstrated that high energy diets may affect mammary development and future milk yield potential [3]. Dietary fiber

can affect animal metabolism, ruminal fermentation and feeding behavior. Previous studies have revealed that dietary  $\text{peNDF}_{8.0}$  content had larger effects on apparent nutrient digestibility, chewing activity, eating and 18.0% dietary  $\text{peNDF}_{8.0}$  content is optimal dietary for 8 to 10-month-old Holstein heifers [4]. It is necessary to quantify the physically effective neutral detergent fiber (peNDF) value in dairy cattle production. Xu et al [5] also reported that a dietary carbohydrate balance index (CBI) of 1.86 could improve the potential growth performance of heifers and promote the maintenance of healthy rumen fermentation, where the computational formula  $\text{CBI} = \text{peNDF}/\text{RDS}$ , where RDS refers to ruminally degradable starch.

Nowadays, most researchers recognize the importance of glucose for ruminants [6]. To assess the adequacy of energy required by dairy cows, the concept of metabolizable glucose (MG) proposed by Lu [7,8] has received increasing attention because it made ruminant glucose nutrition research reach the organism metabolism level, and supplied the specific method for resolving the problems of energy metabolism and substance metabolism. The MG is defined as total amount of digestible glucose of feedstuff origin that can be provided for further use in metabolism, after digested and absorbed by ruminants. The MG in dairy cows has been intensively investigated recently. Researches such as that conducted by Song et al [9] showed that production performance and physiological indices of the Holstein cow under heat stress can be regulated by the MG in diet; it can significantly affect milk yield and feed intake of dairy cow, when the content of MG was 99.27 g/kg. MG could effectively increase the growth rate of replacement heifer and 114 g/kg was the most suitable MG content in diet [10].

Energy efficiency and protein utilization will be improved and the organisms will maintain nitrogen balance if appropriate content of MG is provided. Either inadequate or excessive peNDF in the diet showed negative influence on heifers [4]. MG and peNDF are regarded as the new indexes which amalgamate information on both the apparent nutrition and the regulation of nutrient metabolism. However, limited information is available documenting the influence of interaction between peNDF and MG on heifers, and the most suitable concentration of peNDF/MG in 8 to 10-month-old dairy heifer diets is still uncertain. To find an optimal balance of  $\text{peNDF}_{8.0}/\text{MG}$  content in the diets of dairy heifers is critical to achieve the rearing targets for nutrient utilization, optimal digestion, body size and improved productivity. The optimal  $\text{peNDF}_{8.0}/\text{MG}$  content which we hypothesized in diets would benefit ruminal fermentation, body growth of heifers and improve feed digestion and efficiency. In this investigation, the aim was to assess the different dietary  $\text{peNDF}_{8.0}/\text{MG}$  for growing replacement heifers and to determine the optimal dietary  $\text{peNDF}_{8.0}/\text{MG}$  content.

## MATERIALS AND METHODS

### Experimental design

Animal care and procedures were approved under established standards by the Committee of Yangzhou University on Animal Care and Ethics (No.201406118). The experiment was designed in random block design. Fifteen healthy Holstein heifer calves with similar genetic merit, initial age ( $7.2 \pm 0.18$  months, mean  $\pm$  standard deviation [SD]) and bodyweight ( $256 \pm 12.89$  kg, mean  $\pm$  SD) were randomly assigned to three treatments with five replicates. The experiment was conducted for 40-d and the heifers were given a 15-d adaptation period before exposure to the experimental treatment. The heifers were housed in individual tie stalls, and they were fed one of three diets, which were chemically identical but included different ratios of  $\text{peNDF}/\text{MG}$ , as shown in Table 1. Total mixed

**Table 1.** Composition, particle-size distribution and nutrient levels of the experimental diets

Item	Treatment		
	A(1.46)	B(1.74)	C(2.08)
Ingredients of DM (%)			
Wild ryegrass hay	32.00	32.00	32.00
Corn silage	20.00	20.00	20.00
Corn	30.00	22.00	15.00
Soybean meal	8.00	8.00	7.00
Wheat bran	3.50	8.00	13.00
Distiller dried grains with solubles (DDGS)	0.00	7.50	8.50
Fatty acid calcium	0.00	0.00	2.00
CaHPO <sub>4</sub>	0.50	0.50	0.50
NaHCO <sub>3</sub>	0.50	0.50	0.50
Limestone	0.50	0.50	0.50
NaCl	0.50	0.50	0.50
Premix <sup>1)</sup>	0.50	0.50	0.50
Corn gluten meal	4.00	0.00	0.00
Total	100.00	100.00	100.00
Nutrient levels			
NE <sub>L</sub> (MJ/kg) <sup>2)</sup>	6.65	6.67	6.70
Crude protein (CP)	13.41	13.31	13.43
Starch	27.31	25.77	23.25
Neutral detergent fiber (NDF)	36.36	39.51	41.15
Acid detergent fiber (ADF)	20.05	21.32	21.89
Calcium (Ca)	0.57	0.58	0.60
Phosphorus (P)	0.35	0.41	0.40
Metabolizable glucose (MG) <sup>3)</sup>	12.41	11.35	9.88
DM content of matter in different sieve layer			
$\text{peNDF}_{8.0}$	18.18	19.76	20.58
$\text{peNDF}_{8.0}/\text{MG}$	1.46	1.74	2.08

<sup>1)</sup> The premix was provided with the following per kg of diets: Cu 10 mg, FeSO<sub>4</sub>·H<sub>2</sub>O 24 mg, Zn 38 mg, Co 0.0024 mg, Se 0.0016 mg, Vit A 12,000 IU, Vit D<sub>3</sub> 10,000 IU, Vit E 25 mg, nicotinic acid 36 mg, choline 1,000 mg.

<sup>2)</sup> NE<sub>L</sub>, net energy for lactation (MJ/kg) = 0.5501 × digestible energy (DE; MJ/kg) - 0.3958.

<sup>3)</sup> The MG levels of the three diets were weighted data results, they were calculated based on the MG levels of the ingredients.

rations were fed twice daily at 0700 h and 1700 h respectively to ensure *ad libitum* consumption. Water was available *ad libitum* to the heifers through a water bowl in each stall. The volume of feed allowed for at least a residual of 10%.

### Feeds and diets

The diets were formulated to meet the nutrient requirements of heifers according to NRC [11]. Treatment diets consisted of the following three levels:  $\text{peNDF}_{8,0}/\text{MG} = 1.46$  (Treatment A),  $\text{peNDF}_{8,0}/\text{MG} = 1.74$  (Treatment B) and  $\text{peNDF}_{8,0}/\text{MG} = 2.08$  (Treatment C). The composition and nutrient levels of the experimental diets are shown in Table 1. Wild ryegrass hay was chopped into length of 7 cm using a chaff cutter (model 40B, Xingda Machine Com. Ltd., Zhengzhou, China). The peNDF content was measured by using a Penn State Particle Separator (Anscitech Company, Wuhan, China) [12] and the specific operation steps refer to the reference [13]. For the pef, the proportion of particles larger than 8 mm and less than 19 mm was considered to be  $\text{pef}_{8,0}$  as described in Lammers et al [14]. The  $\text{peNDF}_{8,0}$  content of the ration was calculated by multiplying the neutral detergent fiber (NDF) percentage of the mixed ration by  $\text{pef}_{8,0}$ . MG was calculated by the formula:  $\text{MG} = \text{POEG} + \text{BSEG} = 0.09 \times \text{K}_1 \times \text{Pr} + 0.9 \times \text{K}_2 \times \text{BS}$ . Where POEG refers to the glucose that was generated from propionic acid in the rumen, BSEG refers to the glucose provided by ruminant bypass starch,  $\text{K}_1$  refers to the absorptivity of propionic acid in rumen, Pr refers to the yield of propionic acid in rumen,  $\text{K}_2$  is the absorptivity of ruminal bypass starch in the intestine and BS refers to the amount of ruminal bypass starch.

### Growth-performance measurements and body condition

All heifers were weighed on a platform scale and withers height, body length, heart girth and abdominal girth were measured with a Biltmore stick and tape, after adaptation, at the beginning (the first day) and on the last day (the fortieth day) of the test. The heifers were driven to the fixed bar for measurements before morning feeding. Body condition score (BCS) was also evaluated on the last day of the test and the sample was rated based on a 5-point scoring scheme.

### Blood biochemical indexes

A blood sample of 10 mL was taken from each heifer from the caudal vein before the morning feeding at the end of test (40 days); 1,300×g centrifugal with 15 min prepared serum (stored at  $-20^\circ\text{C}$ ). Serum samples were tested with automatic chemistry analyzer (SINNOW DH-364), and measuring indexes included: total serum protein (TP), albumin (ALB), glucose (GLU), urea nitrogen (UN), total cholesterol (CHOL), high-density lipoprotein (HDL) and low-density lipoprotein (LDL). Then blood samples were taken every 2 h for a total of six times to analyze the change of blood hormone content

with measuring indexes including: insulin (INS), growth hormone (GH), insulin-like growth factor-1 (IGF-1), prolactin (PRL).

### Rumen fermentation

Rumen fluid were sampled 2 h after the morning feeding by an esophageal tube vacuum pump sampling device (Anscitech Company, Wuhan, China) on the three consecutive days (Days 38 to 40) of the experiment. Approximately 200 mL of rumen fluid was sampled after removing saliva from the top of the sampling bottle and then filtered through 4-layer cheesecloth to obtain the strained ruminal fluid. After collection, the ruminal pH was measured immediately with a hand-held electrode (model B-4, Shanghai Chemical Company, Shanghai, China) and two subsamples were preserved. To one subsample, 0.2 mL of 50% (vol/vol) sulfuric acid was added per 10 mL of rumen fluid for subsequent ammonia-nitrogen ( $\text{NH}_3\text{-N}$ ) analysis [15]. For the other subsample, 1 mL of 25% metaphosphoric acid was added per 5 mL of rumen fluid for volatile fatty acid analysis [16]. All samples were stored at approximately  $-20^\circ\text{C}$ .

### Feed intake and digestibility of nutrients

During the experimental period, feed offered and refusals were recorded before the feeding every day. The intake of each individual was calculated as the difference between the dry matter (DM) amount of offered feed and the refused. Fecal grab samples were collected 3 times daily from d 30 to 34 of the experimental period, the fecal samples were pooled by combining approximately 100 g of feces from each time point. Apparent nutrient digestibility was measured according to method of 4 N HCl insoluble ash [17]. The DM content of samples was determined by drying at  $135^\circ\text{C}$  for 3 h [18]. Crude protein was measured with a FOSS N Analyzer (KT260, Foss Co., Hilleroed, Denmark). The NDF and acid detergent fiber (ADF) contents were sequentially analyzed by an Ankom Fiber Analyzer (Ankom Technology, Fairport, NY, USA).

### Statistical analysis

All the data was built in Excel (Microsoft Office, version 2010), and all statistical analyses were performed using the SAS program (SAS Institute, Cary, NC, USA).

Using PROC MIXED of SAS, the experimental data were analyzed in a randomized trial design by using the following model:  $Y_{ijk} = \mu + A_i + M_j + \varepsilon_{ijk}$ ;  $Y_{ijk}$  is the response variable,  $\mu$  is the grand mean,  $A_i$  is the random effect of the heifer ( $i = 1 - 15$ ),  $M_j$  is the fixed effect of diet ( $j = 1 - 3$ ), and  $\varepsilon_{ijk}$  is the random error, which was assumed to be normally distributed.

For all analyses, significance was established as  $p \leq 0.05$ , and a trend was considered to exist if  $0.05 < p \leq 0.10$  using Duncan's new multiple range test. The values are presented as the means and standard errors.

**Table 2.** Effect of different peNDF<sub>8.0</sub>/MG ratios on BW, ADG, and DMI

Item	Treatment			SEM	p-value <sup>1)</sup>
	A(1.46)	B(1.74)	C(2.08)		
Initial BW (kg)	254.60	256.90	256.60	3.33	0.96
Final BW (kg)	294.20	294.00	290.80	3.36	0.91
ADG (kg/d)	0.99 <sup>a</sup>	0.93 <sup>ab</sup>	0.86 <sup>b</sup>	0.02	0.07
DMI (kg/d)	7.10	7.08	7.00	0.07	0.86

peNDF, physically effective neutral detergent fiber; MG, metabolizable glucose; BW, body weight; ADG, average daily gain; DMI, dry matter intake; SEM, standard error of the mean.

<sup>1)</sup> p-values indicate overall diet effects.

<sup>a,b</sup> In the same row, values with different small letter superscripts mean significant difference ( $p < 0.05$ ), while with the same or no letter superscripts mean no significant difference ( $p > 0.05$ ).

## RESULTS

### Body growth and development

No significant difference was found in initial body weight (BW), final BW, and average dry matter intake (Table 2). However, average daily gain (ADG) tended ( $p < 0.10$ ) to decrease with the increasing ratio of peNDF<sub>8.0</sub>/MG. And ADG in Treatment C was 0.86 kg/d, which was significantly ( $p < 0.05$ ) lower than that in Treatment A.

As shown in Table 3, different peNDF<sub>8.0</sub>/MG levels in the diet had no obvious ( $p > 0.05$ ) influence on the change of withers height, body length, paunch girth and BCS. But the strength of growth from heart girth, paunch girth, body length and withers height increased successively and remarkable difference was found in heart girth ( $p < 0.05$ ).

### Blood biochemical indexes

*Serum biochemical parameters:* No significant differences ( $p >$

**Table 4.** Effect of different peNDF<sub>8.0</sub>/MG ratios on serum biochemical parameters of heifers

Items	Treatment			SEM	p-value <sup>1)</sup>
	A(1.46)	B(1.74)	C(2.08)		
TP (g/L)	57.54	55.72	58.02	0.84	0.53
ALB (g/L)	38.06	37.28	37.92	0.24	0.39
Crea ( $\mu$ mol/L)	182.84	182.06	187.02	2.99	0.79
Glu (mmol/L)	5.57	5.59	5.35	0.07	0.27
FFA (ng/mL)	610.15	551.55	600.29	14.57	0.47
HDL (mmol/L)	3.34 <sup>a</sup>	2.66 <sup>ab</sup>	2.44 <sup>b</sup>	0.16	0.04
LDL (mmol/L)	1.39	1.16	1.04	0.89	0.27
TG (mmol/L)	0.33 <sup>b</sup>	0.32 <sup>b</sup>	0.43 <sup>a</sup>	0.02	0.04
CHOL (mmol/L)	2.57 <sup>b</sup>	2.64 <sup>ab</sup>	3.16 <sup>a</sup>	0.11	0.05
UN (mmol/L)	3.83 <sup>b</sup>	4.36 <sup>ab</sup>	4.82 <sup>a</sup>	0.16	0.03

peNDF, physically effective neutral detergent fiber; MG, metabolizable glucose; SEM, standard error of the mean; TP, total serum protein; ALB, albumin; FFA, free fatty acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; CHOL, cholesterol; UN, urea nitrogen.

<sup>1)</sup> p-values indicate overall diet effects.

<sup>a,b</sup> In the same row, values with different small letter superscripts mean significant difference ( $p < 0.05$ ), while with the same or no letter superscripts mean no significant difference ( $p > 0.05$ ).

0.05) were observed in TP, ALB, Crea, Glu, free fatty acid (FFA) and LDL among all groups (Table 4). Another important finding was that the concentration of triglyceride (TG), CHOL and blood urea nitrogen (BUN) improved with the increasing peNDF<sub>8.0</sub>/MG ratio. By contrast, the HDL showed a downward trend with the increasing peNDF<sub>8.0</sub>/MG ratio; The HDL in Treatment A were much higher than those in the other two treatments ( $p < 0.05$ ), but the difference of HDL in Treatment A and B was not obvious ( $p > 0.05$ ).

*Serum hormones:* From Figure 1, it is observed that the mean of INS, IGF-1, GH, and PRL offered a descending ten-

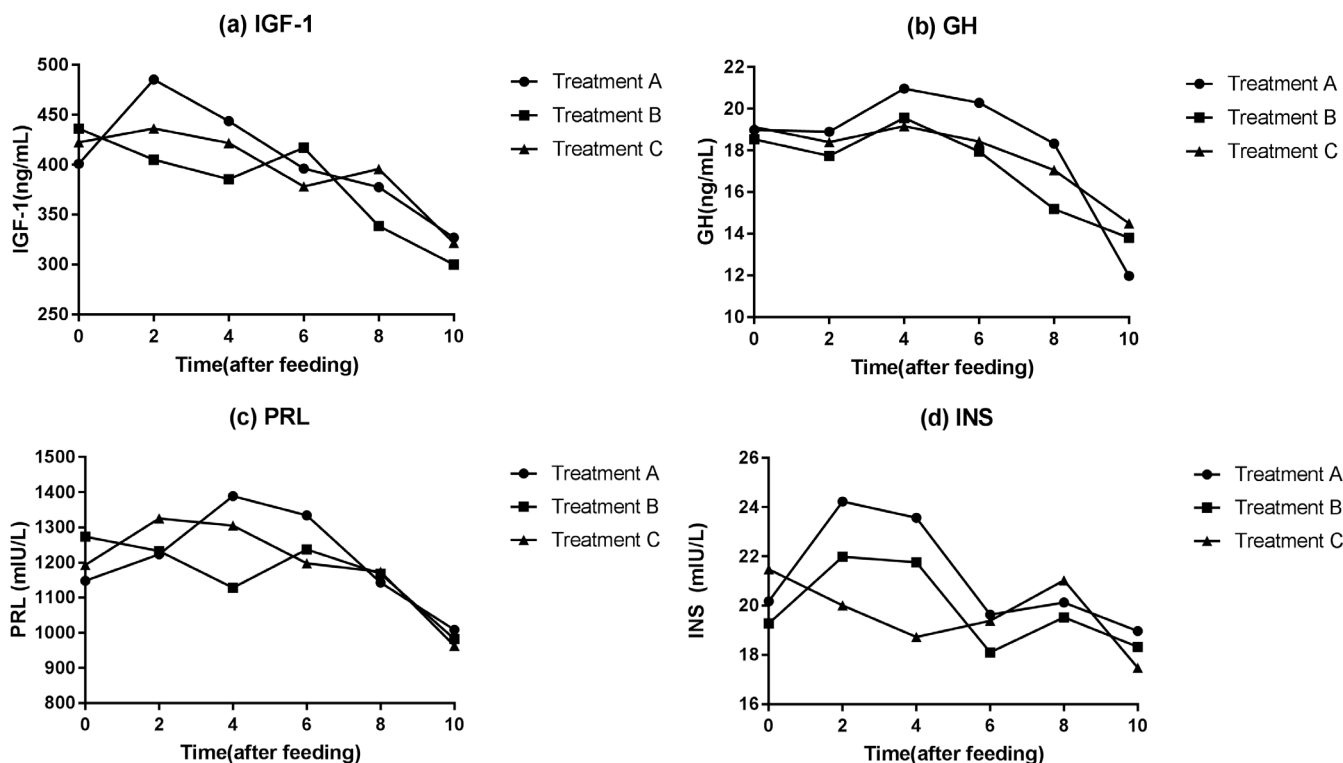
**Table 3.** Effect of different peNDF<sub>8.0</sub>/MG ratios on the body size and body condition score of heifers

Items	Treatment			SEM	p-value <sup>1)</sup>
	A(1.46)	B(1.74)	C(2.08)		
Withers height, initial (cm)	111.40	113.02	112.00	0.52	0.47
Final (cm)	114.80	116.20	115.60	0.48	0.52
Change (cm/d)	0.09	0.08	0.09	0.03	0.89
Body length, initial (cm)	127.80	129.00	127.60	0.65	0.67
Final (cm)	136.40	136.40	136.20	0.88	1.00
Change (cm/d)	0.22	0.19	0.22	0.07	0.78
Heart girth, initial (cm)	149.60	146.80	146.80	0.67	0.15
Final (cm)	154.80	155.80	155.80	0.82	0.87
Change (cm/d)	0.13 <sup>b</sup>	0.22 <sup>a</sup>	0.22 <sup>a</sup>	0.06	0.01
Paunch girth, initial (cm)	180.20	177.00	179.40	0.97	0.40
Final (cm)	189.60	187.40	190.10	1.00	0.54
Change (cm/d)	0.24	0.26	0.27	0.06	0.67
Body condition score (BCS)	2.70	2.80	2.80	0.07	0.80

peNDF, physically effective neutral detergent fiber; MG, metabolizable glucose; SEM, standard error of the mean.

<sup>1)</sup> p-values indicate overall diet effects.

<sup>a,b</sup> In the same row, values with different small letter superscripts mean significant difference ( $p < 0.05$ ), while with the same or no letter superscripts mean no significant difference ( $p > 0.05$ ).



**Figure 1.** Effect of different peNDF<sub>8,0</sub>/MG ratios on serum hormone at different time points of feeding heifers. (a) IGF-1, p = 0.27; (b) GH, p = 0.36; (c) PRL, p = 0.85; (d) INS, p = 0.36. p-values indicate overall diet effects. peNDF, physically effective neutral detergent fiber; MG, metabolizable glucose; IGF-1, insulin-like growth factor-1; PRL, prolactin; INS, insulin.

dency from 0 to 10 h after feeding, and the four hormones at different time points unfold a characteristic of pulsed fluctuations. Those values were highest in Treatment A. At the time point 2 h and 4 h, the mean of serum INS concentration in Group A were greater higher (p<0.05) than those in Group C. Meanwhile, it can be found that mean concentration of INS, PRL, IGF-1, and GH were tending towards stability at 0, 6, 8, and 10 h among three treatments. Compared with Treatment C, there was an obvious declining trend of BUN in Treatment A (p<0.05). In addition, a difference was not noted among the various treatments by statistically analyzing.

**Rumen pH and fermentation**

As shown in Table 5, the pH value was not significantly (p> 0.05) enhanced by increasing peNDF/MG ratios, and the ruminal pH levels were above 6.5 in all treatments. The results showed that NH<sub>3</sub>-N concentration in Treatment A was substantially below in Treatment C. Moreover, no significant (p> 0.05) effect of the treatments was observed on the total VFA concentration, and the percentages of acetic acid, butyric acids and branched-chain fatty acids remained unchanged among the treatments. The proportion of propionate acid was significantly decreased by an increasing peNDF<sub>8,0</sub>/MG ratio, and the highest value of propionate was obviously in Treatment A (p< 0.05), as well as acetate to propionate ratio (A/P) and non-

glucogenic to glucogenic volatile fatty acid (NGR).

**Nutrient digestibility**

Digestibility of DM, crude protein (CP), ADF, and NDF

**Table 5.** Effect of different peNDF/MG ratios on characteristics of rumen fermentation of heifers

Items	Treatment			SEM	p-value <sup>1)</sup>
	A(1.46)	B(1.74)	C(2.08)		
pH	6.51	6.53	6.66	0.05	0.48
NH <sub>3</sub> -N (mg/100 mL)	14.25 <sup>a</sup>	11.76 <sup>ab</sup>	8.88 <sup>b</sup>	0.78	0.01
Total VFA (mmol/L)	85.50	71.84	78.79	3.26	0.25
Acetate (%)	68.75	69.56	69.34	0.27	0.49
Propionate (%)	18.59 <sup>a</sup>	17.58 <sup>b</sup>	17.74 <sup>b</sup>	0.20	0.07
Butyrate (%)	9.13	9.65	9.74	0.18	0.36
BCFA <sup>2)</sup> (%)	2.59	2.41	2.32	0.08	0.40
Acetic/propionate	3.70 <sup>b</sup>	3.97 <sup>a</sup>	3.91 <sup>a</sup>	0.06	0.04
NGR <sup>3)</sup>	4.50 <sup>b</sup>	4.89 <sup>a</sup>	4.82 <sup>a</sup>	0.07	0.03

peNDF, physically effective neutral detergent fiber; MG, metabolizable glucose; SEM, standard error of the mean; VFA, volatile fatty acid.

<sup>1)</sup> p-values indicate overall diet effects.

<sup>2)</sup> BCFA is branched-chain fatty acids (isovaleric acid and isobutyric acid).

<sup>3)</sup> NGR is the ratio of non-glucogenic fatty acid to glucogenic fatty acid, the method for its calculation is  $NGR = (\text{acetate} + 2 \times \text{butyrate} + \text{valeric}) / (\text{propionate} + \text{valeric})$ .

<sup>a,b</sup> In the same row, values with different small letter superscripts mean significant difference (p<0.05), while with the same or no letter superscripts mean no significant difference (p>0.05).

**Table 6.** Effect of different peNDF<sub>8,0</sub>/MG ratios on nutrient digestibility of heifers (%)

Items	Treatment			SEM	p-value <sup>1)</sup>
	A(1.46)	B(1.74)	C(2.08)		
DM	75.57 <sup>a</sup>	63.63 <sup>b</sup>	58.11 <sup>c</sup>	3.05	<0.01
CP	78.32 <sup>a</sup>	72.66 <sup>b</sup>	63.40 <sup>c</sup>	2.51	<0.01
NDF	65.82 <sup>a</sup>	57.87 <sup>b</sup>	57.52 <sup>b</sup>	3.44	0.01
ADF	60.32 <sup>a</sup>	50.46 <sup>b</sup>	51.00 <sup>b</sup>	3.42	0.01

peNDF, physically effective neutral detergent fiber; MG, metabolizable glucose; SEM, standard error of the mean; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.

<sup>1)</sup> p-values indicate overall diet effects.

<sup>a,b,c</sup> In the same row, values with different small letter superscripts mean significant difference ( $p < 0.05$ ), while with the same or no letter superscripts mean no significant difference ( $p > 0.05$ ).

decreased ( $p < 0.05$ ) with increasing peNDF<sub>8,0</sub>/MG as demonstrated in Table 6. A significant difference was observed in the apparent digestibility of DM and CP ( $p < 0.05$ ). The highest apparent digestibility of DM and CP was observed in Treatment A ( $p < 0.05$ ), and so were nutrient digestibility of NDF and ADF.

## DISCUSSION

Dairy heifer growth, body size and ADG at the first calving can affect future milk yield potential, they are regarded as an important management target for the future of heifers [19]. Fiber content and energy level of the diet are two important factors which we should take into consideration during the process of dairy cows production. It has been demonstrated that the peNDF content in diets had no significant effect on BW, DG, and DMI of heifer herds [4]. In addition, some studies showed that the MG level in the diet had no significant effect on the BW and DMI, but it may promote the daily gain of heifer herds [10]. The present study obtained similar results and there was a tendency to decrease the ADG of the test herds. The reason for decreasing ADG might be explained based on the fact that different MG resulted in different energy intake. It was also found in Ge's [20] research that the ADG improved dramatically with increasing MG content. In this study, heifers had best growth performance when peNDF<sub>8,0</sub>/MG ratio in the diet was 1.46, and we predict that the weight of the heifers would be ranged from 380 kg to 400 kg at the age of 13 months according to the tendency.

Cows with smaller udders and greater abdominal circumference had higher lactation rates [21]. We found lower peNDF<sub>8,0</sub>/MG ratio in diet can significantly reduce udder development, which was beneficial to milk production. In this study, the BCS of heifers ranged from 2.7 to 2.8, which was consistent with the recommendations [22]. It also implied that the heifers were in good condition. The content levels of HDL, LDL, CHOL, and TG reflected the absorption, metabolism

and utilization of lipids; the lower the TG content, the higher utilization of fat. The TG content in Treatment A was low. It illustrated that lower peNDF<sub>8,0</sub>/MG ratio is conducive to decrease serum level of lipids, and overweight could also be avoided. The main physiological functions of HDL is to transport CHOL to cells throughout the body, and finally to the liver for bile acid synthesis [23]; LDL enriched CHOL will lead to atherosclerosis if the content is too high [24]. The results of this experiment showed that the concentration of HDL and CHOL had inverse relation in the same treatment, which indicated that lower peNDF<sub>8,0</sub>/MG ratio could promote CHOL metabolism and reduce CHOL deposition in the body thereby improving the content of HDL. FFA has been identified as one of major contributing factors for energy balance of the body [25]. When the energy cannot meet metabolic need, the result will be elevated FFA in blood [26]. During the experiment, the results showed that FFA was not affected by peNDF<sub>8,0</sub>/MG ratio in the diet; this is probably a result of the same energy level being provided. It has been demonstrated that elevated serum BUN level results in strengthening protein decomposition; but there will be an increased protein synthesis and improved utilization of nitrogen, if protein decomposition has declining trend. In this experiment, the declining trend of BUN indicated that lower peNDF<sub>8,0</sub>/MG ratio (Treatment A) may promote the utilization of nitrogen and deposition of protein synthesis.

The growth of animals is regulated by hormones and growth factors. INS plays an important role in promoting the storage of glycogen TG, reducing the glucose content and facilitating protein synthesis. Data from previously published papers have identified a significant positive correlation between INS content and serum glucose level in pregnant sheep [27]. The level of INS significantly increased in Treatment A at 2 and 4 h after feeding; because high MG diet contributed to the elevated level of glucose, then endocrine cells produced more INS to regulate hormone balance in the body. Radcliff [28] reported that giving exogenous GH to heifer herds increased ADG, it also can prompt feed conversion rate and reduced body fat deposition rate. GH exerts some of its action via IGF-1, and consistent with this, the concentration of IGF-1 in blood is increased by GH treatment [29]. The current study found that the concentration of GH and IGF-1 were highest in Treatment A for a period of time after feeding, and the concentration of IGF-1 tended to increase at 2 h in Treatment A. The change of ADG was also consistent with the above results. PRL is a hormone secreted by the pituitary gland, which can promote growth, development and lactation. The results showed that the level of PRL in Group A was significantly higher than that in the Group B at 4 h after feeding, but there were no difference at the rest of the time points. It's probably affected by different diets treatment, but the relationship of the two factors needs further research.

Ruminal pH is a fundamental index used to evaluate rumen fermentation conditions. It reflects the balance between organic acid production in the rumen and acid removal through neutralization and absorption within the rumen [4]. The rumen microbe growth depends on carbohydrates supply in diet; it will also contribute to the occurrence of rumen acidosis, if ruminants are given too much fermentable carbohydrates [30]. In this study, the ruminal pH in heifers ranged from 6.5 to 6.7, which was far from the range of 5.2 to 5.6 (SARA). It indicated that the diets we provided had an appropriate ratio of peNDF<sub>8,0</sub>/MG content and there was a dynamic balance between rumen microorganisms, host and also between microbes. Ruminal NH<sub>3</sub>-N is the degradation product of protein and non-protein N. And it is used for bacterial protein synthesis. The NH<sub>3</sub>-N concentration in Treatment A was much higher than that in Treatment C, although the CP content in three treatments was the same. The reason for decreasing NH<sub>3</sub>-N concentration could be explained based on the fact that microbes quickly multiply and the NH<sub>3</sub>-N was used to synthesize the microbe protein.

Ruminal VFA reflects an integrated balance of organic acid that could be affected by the production, absorption, and turnover rate in the rumen [4]. Previous studies have revealed that the concentration of total VFA is closely correlated with the combined effect of coarse fodder and MG, but the single factor of MG has no effect on total VFA [31]. The current study obtained similar results that the total VFA had no obvious change with the decreasing MG level. Acetate acid would be produced by fiber degradation in rumen, whereas starch degradation produces more propionic acid. Firkins et al [32] observed that the acetate to propionate ratio correlated positively with forage NDF and negatively with nonstructural carbohydrates in the diet of dairy cows. Data from current study illustrates that the differences of molar ratio between acetic acid and butyric acid in rumen fluid among each treatments were not significant. The NDF content in Treatment A was low, but there was a high digestion rate of NDF. The molar ratio of propionate manifested a decreasing tendency with increasing peNDF<sub>8,0</sub>/MG, that was because starch content in the diet was gradually increasing from Group C to Group A. A/P and NGR are two important indicators, which may decide the rumen fermentation pattern [7]. In this experiment, A/P and NGR increased remarkably when peNDF<sub>8,0</sub>/MG was raised. It confirmed that the ratio of dietary peNDF<sub>8,0</sub>/MG could have a significant effect on the rumen fermentation pattern.

To a large extent, nutrient digestibility would be influenced by the ability of ruminal degradation. Several studies have revealed that the dietary peNDF content made a significant impact on replacement heifers' nutrient digestibility [4], while the MG content was without influence on the apparent digestibility [10,20]. Heifers were fed the diet with the same peNDF<sub>8,0</sub>,

however, we found that the apparent digestibility changed which were inconsistent with previous studies. This could be due to the content of glucose supplied to the body was reduced, the lower MG content, the less glucose supplied and digestive juices secreted. The activity of rumen microbes could also be influenced accordingly.

## CONCLUSION

During the experiment with dairy heifers, carbohydrate composition was expressed as the ratio peNDF to MG. We recommended a dietary peNDF/MG of 1.46 to promote the maintenance of healthy rumen fermentation and to improve the potential growth performance of 8 to 10 months old heifers. This finding will enhance current knowledge and help establish additional alternative dietary recommendations for dairy replacement heifers.

## CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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