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RESEARCH ARTICLE

Effects of Different Dietary Energy and Rumen-Degradable Protein Levels on Rumen Fermentation, Nutrients Apparent Digestibility and Blood Biochemical Constituents of Chinese Crossbred Yellow Bulls

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ABSTRACT

The experiment was conducted to evaluate the effects of two dietary energy levels (TDN: 70 and 76% DM) and two rumen-degradable protein levels (RDP: 7.7 and 9.4% DM) on rumen fermentation, nutrients apparent digestibility and blood biochemical constituents of Chinese crossbred yellow bulls. Four ruminally-fistulated Charolais×Nan yang yellow bulls, about 540±23kg live weight, were randomly assigned to a 2×2 factorial arrangement in a 4×4 Latin Square design to receive four dietary treatments. The treatments were as follows: low energy and high protein (LEHP; TDN: 70%, RDP: 9.4%), high energy and high protein (HEHP; TDN: 76%, RDP: 9.4%), low energy and low protein (LELP; TDN: 70%, RDP: 7.7%) and high energy and low protein (HELP; TDN: 76%, RDP: 7.7%). Ruminal pH and total volatile fatty acids were not different among treatments. The acetate concentration was lower and propionate concentration was greater ($P<0.01$) for bulls fed HE diet compared with LE diet. The higher ammonia nitrogen (NH_3–N) concentration was found for bulls fed HP diet compared to bulls fed LP diet ($P<0.01$). Total apparent digestibility of dry matter (DM), crude protein (CP), organic matter (OM) and N utilization were greater for bulls fed HE vs LE diet ($P<0.05$). Bulls fed HP diet had increased N retention than those fed LP diet ($P<0.05$). Blood biochemical constituents were not different among all dietary treatments, except plasma urea nitrogen (PUN) which was higher in HP dietary treatment ($P<0.05$). These findings suggest that high energy and high protein (energy: 76%, RDP: 9.4%) treatment is the best for high performance of yellow bulls without affecting their health.

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INTRODUCTION

A complex inter-relationship exists among dietary energy, protein and the amount of protein that could be utilized by animals. High concentrate diets based on cereal grains can increase growth rate and improve production efficiency of beef cattle (Brown *et al.,* 2006). High energy diets contribute to microbial protein synthesis (Bach *et al.,* 2005), increasing the supply of microbial protein to small intestine. Therefore, increasing dietary energy content may increase rumen degradable protein (RDP) requirements.

It is uneconomical to over feed energy and protein. Overfeeding energy in terms of high concentrate diets results in accumulation of volatile fatty acids (VFA) in the rumen and decreases rumen pH. The lower pH especially below 5.6, is detrimental for animal health and production (Krause and Oetzel, 2006). Moreover, overfeeding protein could result in excessive N, which pollutes the environment (Varel *et al.,* 1999).

The main objective of this experiment was to quantify the dietary concentrations of energy and protein that would minimize N excretion without depressing animal health. Experiment was designed to evaluate the effects of two levels of dietary energy and rumen-degradable protein (RDP) on rumen fermentation, nutrients apparent digestibility and blood biochemical constituents of Charolais×Nan yang yellow bulls.

MATERIALS AND METHODS

Animals, feeds and management: Four ruminally-fistulated Charolais×Nan yang yellow bulls, average 540±23kg live weight, were selected. Bulls were placed in a 2×2 factorial arrangement using 4×4 Latin Square design to evaluate the effects of two dietary energy levels (TDN: 70 and 76% DM) and two rumen degradable protein levels (RDP: 7.7 and 9.4% DM) on rumen fermentation, nutrients apparent digestibility and blood biochemical constituents. Rumen-undegradable protein (RUP) contents were similar in all treatments diet. Treatment groups were as follows: low energy and high protein (LEHP; TDN: 70%, RDP: 9.4%), high energy and high protein (HEHP; TDN: 76%, RDP: 9.4%), low energy and low protein (LELP; TDN: 70%, RDP: 7.7%) and high energy and low protein (HELP; TDN: 76%, RDP: 7.7%). Each experimental period lasted 16 days which included 13 days for dietary treatment adaption and three days for sample collection. Each bull was kept in individual sheltered pens of approximately 20m^2. Bulls were fed twice daily at 06:00 and 18:00 and allowed to drink water freely. Dietary ingredient and nutrition levels are shown in Table 1.

Table1: Dietary ingredient and nutrition levels of different treatment (feed) groups

Item	HP		LP	
	LE	HE	LE	HE
Ingredient (% DM)				
Ground corn	30.32	47.36	35.58	52.48
Soybean meal	14.40	16.76	8.45	10.64
Cottonseed meal	3.13	0.00	3.00	0.00
Wheat bran	19.40	13.00	20.22	14.00
Limestone	1.00	1.00	1.00	1.00
Salt	1.05	1.20	1.05	1.20
Premix[1]	0.70	0.80	0.70	0.80
Rice straw	30.00	20.00	30.00	20.00
Nutrient level (% DM)				
DM	86.94	86.70	86.80	86.56
CP	14.05	14.00	11.96	11.90
RDP	9.37	9.35	7.75	7.73
RUP	4.69	4.65	4.20	4.27
TDN	70.10	75.93	70.11	76.34
NDF	33.59	25.53	33.56	25.58
ADF	18.27	13.10	17.98	12.85
NSC	38.90	47.74	41.44	50.22
Ca	0.44	0.43	0.43	0.41
P	0.38	0.33	0.37	0.32

1. Vitamin and mineral premix contained per kilogram of DM: 3150IU Vitamin A, 1550 IU Vitamin D, 35.5 IU Vitamin E, 90 mg Fe, 100 mg Zn, 40 mg Mn, 11.5 mg Cu,0.71 mg I, 0.60 mg Se, 0.90 mg Co, 30 g/1000 kg Monensin.

Sample Collection and Analyses

Ruminal contents: During the sample collection period, ruminal fluid was collected every day at 0, 2, 4, 6, 8, 10 and 12 h after morning feeding to determine ruminal pH, the concentrations of VFA and NH_3-N. Samples were collected from the rumen and squeezed through four layers of cheesecloth. Ruminal pH was measured immediately with a portable pH meter (HJ-90B), then samples were centrifuged at 5,000 rpm /min for 20 min, supernatant liquid was put into two 10 mL tubes. One subsample was analyzed for ammonia N by spectrophotometer (UV-1700, Shimazu Corporation) following the methods described by Broderick and Kang (1980). The other subsample was used for VFA analysis by gas chromatograph (GC-2014, Shimazu Corporation) with a capillary column (Agilent HP-INNOWAX, 30 m long, 0.32 mm diameter, 0.50 μm film) using the method of Kim *et al.* (2013).

Feed, Fecal and urine samples: Feeds, refusals were weighted and samples were collected every day during the sample collection period. At the same time, fecal and urine of each individual bull was collected using total collection method. Fecal weigh was recorded and the sample (approximately 300g on a wet weight basis) was mixed with 75 mL 10% tartaric acid, then dried at 60˚C for 48h and grounded by 1-mm screen. Urine was put into containers having 200 mL 10% sulfuric acid.

Feed, refusal and fecal samples were used to analyze DM, CP and ash by using standard methods of AOAC (2000). NDF and ADF were determined using filter bags and fiber analyzer equipment (Fiber Analyzer, Ankom Technology, Macedon, NY) following a modification of the procedure of Van Soest *et al*. (1991). Apparent digestibility was determined by the formula:
(Nutrients in feed intake - nutrients in fecal) / Nutrients in feed intake × 100%. Total N of feed, refusal, fecal and urine were determined by the Kjeldahl method, as described by AOAC (2000) to calculate the N retention and utilization of the bulls.

Blood biochemical constituents: On the final day of the experiment trail, blood samples (about 10 mL) were collected from the jugular vein in tubes containing 12 mg of EDTA at 0,6,12 h after the morning feeding, plasma was separated by centrifugation at 5,000 rpm /min for 20 min at 4°C within 1 h of collection. The supernatant was collected and stored at -20°C until analysis. Blood biochemical constituents were determined by using Hitachi 7020 automated biochemistry analyzer (Hitachi Co., Tokyo, Japan). Glucose (Glu), triglycerides (TG), total proteins (TP) and plasma urea nitrogen (PUN) concentration were determined using the test kit with the methods of oxidase, GPO/PAP, biuret and Urease UV Liquid respectively.

Statistical analysis: Statistical analysis of the data was conducted using the PROC MIXED procedure of SAS (Version 9.0, SAS Inst. Inc., Cary, NC). Period, energy, RDP and the interaction of energy × RDP were considered as fixed effects, whereas animal was considered as random effect. Differences were declared significant at $P<0.05$, when significance was detected, treatment means were compared by Tukey's Multiple Comparison Test.

Ruminal fermentation characteristics and blood samples collected at different times after feeding were analyzed for repeated measures. Repeated factors included days, sampling time after feeding and the day × time interaction. For every analyzed variable, bull and period nested within treatment was considered as a subject. The covariance structure that yielded the smaller Akaike and Schwarz's Bayesian criterion was considered to be the most desirable for analysis.

RESULTS

Rumen fermentation: The effects of energy and RDP levels on rumen fermentation are shown in Table 2.

Ruminal pH and total VFA concentrations of each treatment at different sampling times are shown in Figure 1 and Figure 2. Ruminal pH, total VFA and butyrate concentrations did not differ according to the treatments, averaged 6.51, 97.20 m*M* and 11.64 m*M*, respectively. Ruminal NH_3-N and isovalerate concentration were greater for bulls fed HP diet compared with LP diet (6.35 vs 4.58 mg/dL; 2.92vs 2.62 m*M*, P<0.01). Bulls fed HE diet had a lower acetate concentration (56.47 vs 61.05 m*M*, P<0.01) and acetate: propionate ratio (2.57 vs 3.08, P<0.01), but a higher propionate (22.36 vs 20.22 m*M*, P<0.01) and isovalerate concentration (2.90 vs 2.64 m*M*, P<0.01) than those fed LE diet. The interaction of dietary energy and RDP levels was significant for isobutyrate, valerate concentrations and the ratio of acetate: propionate (P<0.05).

Nutrients digestibility and nitrogen balance: The effects of energy and RDP levels on feed intake, nutrients apparent digestibility and N metabolism are shown in Table 3. DM and OM intake of bulls were non-different among the treatments. The bulls fed HP diet had a higher CP intake compared to those fed LP diet (1.44 vs 1.22 kg/d, P<0.01). Compared with bulls fed HE diet, the great NDF (3.45 vs 2.60 kg, P<0.01) and ADF (1.86 vs 1.33 kg, P<0.01) intake were found in the bulls fed LE diet. Bulls fed HE diet had increased apparent digestibility of DM (76.0 vs 70.8%, P<0.01), CP (75.2 vs 71.5%, P<0.01), and OM (79.5vs 74.6%, P<0.01) compared with bulls fed LE diet. Apparent digestibility of NDF and ADF were similar among treatments, but they were slightly lower in HE treatments.

N intake and retention were higher as the RDP level increased (P<0.05). Fecal N and urinary N of the bulls were not different among the treatments (P>0.05). N utilization was greater for bulls fed HE diet than those fed LE diet (P<0.05).

Blood biochemical constituents: Effects of energy and RDP levels on blood biochemical constituents are shown in Table 4. Blood plasma Glu, TG and TP concentrations were not affected by energy and RDP levels. PUN concentration increased with increasing RDP level in the diet (3.84 vs 3.25 m*M*, P<0.01).

DISCUSSION

Rumen fermentation: The ruminal pH is a critical parameter directly affecting microbial growth and rumen fermentation. Ruminal microorganisms are well adapted to develop in a pH varying from 5.6 to 7.0 (Hoover and Stokes, 1991). Ruminal pH of 5.6 or below is generally considered the threshold for ruminal acidosis which results in negative effects on normal rumen function. When beef cattle are fed high-grain diet, ruminal pH can range from 5.6 to 6.5, typically around 5.8 to 6.2 (Nagaraja and Titgemeyer, 2007). In the present study, average pH value was above 6.45 in each treatment. Ruminal pH fluctuates considerably in a 12-h period, between 5.98-6.85 which is within the optimum range for microorganisms growth and without risk for rumen acidosis (Fig. 1). The lowest pH value was detected at 4h or 6 h after morning feeding, and was mainly the result of

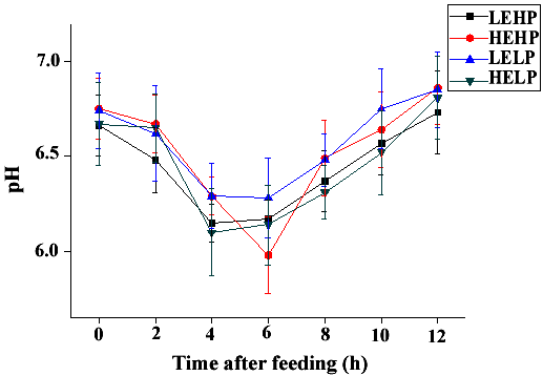

Fig. 1: Ruminal pH value of each treatment at different sampling time after feeding

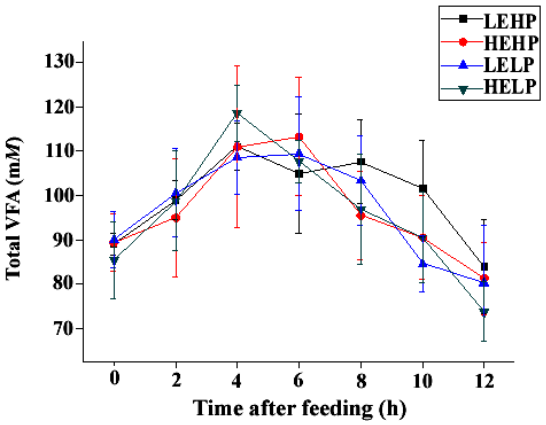

Fig. 2: Ruminal total VFA concentration of each treatment at different sampling time after feeding

the accumulation of total VFAs, as carbohydrate fermentation was the highest during this time (Fig. 2). Figure 1 and Figure 2 show that the pH value increased because of the total VFAs were absorbed by rumen from 6h to 12h, which is in agreement with other researches (Rotger *et al.*, 2006; Whelan *et al.*, 2013).

Ammonia is used for microbial protein synthesis, the optimal level of NH_3-N concentration for efficient digestion is from 5.0 to 30.0 mg/dL (Thao *et al.*, 2014). In the current study, NH_3-N concentrations of the four treatments were between 4.45 and 6.68 mg/dL (Table 2). NH_3-N concentrations in the lower RDP treatments were below 5.0 mg/dL. This might have been caused by the lower dietary RDP level that could not provide enough ammonia in the rumen, by which the efficiency of microbial protein synthesis could be affected.

Total VFAs concentrations were not different among the treatments, but rumen fermentation pattern was not the same. Acetate is the major end product of fiber fermentation and increased acetate concentration was observed in cattle fed higher level of dietary fiber (Felix *et al.*, 2012). On the other hand, the propionate concentration tended to increase with increasing dietary energy level by adding more non-structure carbohydrates (NSC) (Keady and Mayne, 2001). In the current study, HE diet contained more concentrate and less fiber than LE diet, so rumen fermentation pattern was changed. These results are consistent with other researches (Agle *et al.*, 2010b). Isovalerate concentration was greater for bulls fed

Table 2: Effects of energy and RDP level on rumen fermentation index

Item	HP		LP		SEM	RDP	E	E×RDP
	LE	HE	LE	HE				
pH	6.45	6.52	6.51	6.57	0.05	ns	ns	ns
NH_3-N (mg/dL)	6.68[a]	6.01[a]	4.45[b]	4.70[b]	0.32	**	ns	ns
Volatile fatty acids (m*M*)								
Acetate	61.11[a]	56.70[b]	60.99[a]	56.24[b]	1.40	ns	**	ns
Propionate	21.07[ab]	22.26[a]	19.36[b]	22.46[a]	0.59	ns	**	ns
Butyrate	11.69	11.93	11.38	11.55	0.38	ns	ns	ns
Isobutyrate	1.81	1.65	1.52	1.81	0.10	ns	ns	*
Valerate	1.09	1.01	0.99	1.11	0.03	ns	ns	*
Isovalerate	2.82[a]	3.01[a]	2.46[b]	2.78[a]	0.09	**	**	ns
Total VFA	99.59	96.56	96.71	95.95	2.19	ns	ns	ns
Acetate: propionate	2.95[b]	2.59[c]	3.21[a]	2.54[c]	0.08	ns	**	*

Significance: *(P<0.05), **(P<0.01), ns not significant (P>0.05); [a b] Means within same row with the same superscript letter are not significantly different (P>0.05); m*M* = 0.001 mol/L

Table 3: Effects of energy and RDP level on feed intake, nutrients apparent digestibility and N metabolism

Item	HP		LP		SEM	RDP	E	E×RDP
	LE	HE	LE	HE				
Feed intake (kg/d)								
DM	10.26	10.20	10.24	10.16	0.02	ns	ns	ns
CP	1.44[a]	1.43[a]	1.22[b]	1.21[b]	0.01	**	ns	ns
OM	9.31	9.35	9.29	9.30	0.05	ns	ns	ns
NDF	3.45[a]	2.60[b]	3.44[a]	2.60[b]	0.03	ns	**	ns
ADF	1.87[a]	1.34[b]	1.84[a]	1.31[b]	0.03	ns	**	ns
Apparent digestibility (%)								
DM	71.50[ab]	77.00[a]	70.00[b]	75.00[a]	1.44	ns	**	ns
CP	72.85[ab]	77.15[c]	70.06[b]	73.18[ac]	1.55	ns	**	ns
OM	75.63[ab]	80.13[a]	73.63[b]	78.88[a]	1.28	ns	**	ns
NDF	63.50	59.00	61.50	51.50	4.70	ns	ns	ns
ADF	60.00	57.50	55.50	54.00	4.59	ns	ns	ns
N metabolism (g/d)								
N intake	230.64	228.48	195.95	193.45	0.53	**	ns	ns
Fecal N	62.62	56.02	54.76	51.88	2.56	ns	ns	ns
Urinary N	78.08	75.74	69.01	62.25	3.02	ns	ns	ns
N retention	89.95[a]	96.72[a]	72.18[b]	79.31[b]	2.84	*	ns	ns
N utilization (%)	39.00[ab]	42.33[b]	36.83[a]	41.00[b]	1.95	ns	*	ns

Significance: *(P<0.05), **(P<0.01), ns not significant (P>0.05); [a b] Means within same row with the same superscript letter are not significantly different (P>0.05).

Table 4: Effects of energy and RDP level on blood biochemical constituents

Item	HP		LP		SEM	RDP	E	E×RDP
	LE	HE	LE	HE				
TP (g/L)	77.98	78.20	77.29	77.85	1.95	ns	ns	ns
Glucose (m*M*)	3.66	3.65	3.64	3.66	0.10	ns	ns	ns
TG (m*M*)	0.13	0.12	0.13	0.11	0.01	ns	ns	ns
PUN (m*M*)	3.98[a]	3.69[b]	3.41[c]	3.08[d]	0.06	**	**	ns

Significance: *(P<0.05), **(P<0.01), ns not significant (P>0.05); [a b]Means within same row with the same superscript letter are not significantly different (P>0.05).

HP diet compared to those fed LP diet. Isovalerate is mainly built up from the degradation products of the amino acids (Scott *et al.,* 2013). That is why higher isovalerate concentration was found in HP treatment.

Effect of different treatments on nutrients digestibility: Digestibility is usually determined by the absorption and the passage rate of feed in gastrointestinal tract. Passage rate is always positively related to the DMI (Clark *et al.,* 1992). DM and OM intake were similar among the treatments, increasing energy level improved the utilization of diet. Pereira *et al*. (2008) and Benchaar *et al*. (2012) reported that total-tract apparent digestibility of DM and OM was increased by increasing concentrate proportion in the diet. The digestibility of NDF and ADF had the tendency to decrease as the energy level increased. It was similar to what was found by Bailey *et al*. (2012). One reason for this might be the decrease of ruminal pH value, because cellulolysis would be seriously inhibited when ruminal pH is below 6.2 (Anantasook *et al.,* 2013). Reduction in ruminal pH below 6.3 in dairy cows resulted in a 3.6 percentage unit decline in ADF digestion per 0.1 pH unit decrease (Erdman, 1988). Another explanation might be the competitive inhibition among microorganism, the ruminal microorganisms use non-structure carbohydrate first, which inhibits the growth of cellulolytic bacteria. So the decreased number of cellulolytic bacteria leads to the reduction of the fiber digestion. In the present study, pH values were not different among treatments, so the digestibility of NDF and ADF can be explained by the inhibition theory among microorganisms.

RDP is essential for microbial growth, so the different protein levels affect the quantity and activity of the microorganisms, which indirectly influences the nutrients digestibility. Huyen *et al*. (2012) reported that beef cattle fed increasing amounts of mulberry leaf pellet (MUP) showed increased nutrients apparent digestibility. However, other reports found that apparent digestibility of the nutrients was not affected by different levels of protein (Kokkonen *et al.,* 2002; Agle *et al.,* 2010a). Broderick (2003) found that the digestibility of NDF and ADF increased with increasing protein level for dairy cows, but Koster *et al.* (1996) observed no such effects on digestibility. The results in the present study revealed that the RDP level met the needs of microorganisms growth, so dynamic balance between absorption and passage rate was not disturbed.

Effect of different treatments on nitrogen balance: N retention is an important index, which reflects the protein nutrition status of ruminants. Positive nitrogen retention was observed in the present study when animals were fed HP diet. N utilization was increased with increasing energy level. Hoover and Stokes (1991) also reported increased N utilization by increasing the energy level. The possible reason could be the fact that energy of the diet is utilized for microbial protein synthesis, so when energy supply is available, the microbial protein synthesis efficiency is raised, resulting in higher N utilization.

Effect of different treatments on blood biochemical constituents: Blood plasma concentrations of TP, Glu and TG were similar among the treatments. PUN concentration was greater in the higher RDP treatments, because higher RDP treatments produced more NH_3-N and entered into the blood stream, which was utilized by liver to synthesize more urea nitrogen. Hof *et al*. (1997) reported that PUN always has the positive correlation with the protein intake. According to Whitelaw *et al*. (1991), the concentration of PUN was increased when NH_3-N concentration was higher in the rumen.

Conclusion: This study indicated that energy level played an important role on dietary nutrients assimilation. Higher dietary energy and protein concentrations minimize N excretion without having negative effect on animal health. We strongly recommend energy: 76% and RDP: 9.4% for yellow cattle for high performance without affecting their health.

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