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Effects of dietary forage sources on rumen microbial protein synthesis and milk performance in early lactating dairy cows

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ABSTRACT

The objective of this study was to evaluate the effects of dietary forage sources on milk performance, rumen microbial protein synthesis, and N utilization in early lactation dairy cows. Twelve primiparous Chinese Holstein dairy cows (45 ± 6.0 DIM) were used in a 3 × 3 Latin square design. Diets were isonitrogenous and isocaloric, with a forage-to-concentrate ratio of 45:55 [dry matter (DM) basis] and contained similar concentrate mixtures. Different forage sources were then added (on a DM basis): 21% corn silage, 19% corn stover, and 5% alfalfa hay (CS); 19% corn silage, 21% Chinese wild rye hay and 5% alfalfa hay (CWR); or 19% corn silage, 9% Chinese wild rye hay, and 17% alfalfa hay (AH). Each period lasted for 21 d, with the first 14 d for an adaptation period. Dry matter intake was not affected by the source of dietary forage. Milk yield was higher for cows fed AH than those fed CS, with an intermediate value for CWR. Milk protein content was higher in the cows fed AH compared with CWR (3.02 vs. 2.92%), with CS (2.95%) at an intermediate position. The contents of milk fat and lactose were not different among the treatments. However, milk efficiency (milk yield/DM intake) was higher for cows fed AH than those fed CS, with those fed CWR intermediate. Cows fed AH had higher microbial protein yield and metabolizable protein than those fed CS or CWR. The concentrations of urea N in the urine, blood, and milk were decreased for cows fed AH, indicating an increased N conversion. The results indicated that corn stover could replace Chinese wild rye grass in the diets for lactating cows and that a high proportion of alfalfa hay in the diet is beneficial for milk protein production by increasing microbial protein yield. This can be attributed to the improving the supply of rumen-available energy.

Key words: forage, microbial protein, lactation

INTRODUCTION

Forage comprises half or more of the diet of dairy cows, affecting the DMI and thereby affecting the energy intake (Kendall et al., 2009). With the development of herbivorous animals, the gap between forage supply and demand is increasing by 10% annually in China (Li and Wan, 2005). High-quality forages such as alfalfa hay for dairy cows is especially in short supply in China. In 2010, 230 × 10^6 kg of alfalfa hay had been imported from foreign countries, mainly from the United States, whereas only 100 × 10^6 kg was produced domestically (Wang, 2011). At the same time, it is estimated that more than 100 × 10^9 kg of corn stover is generated annually in China (Pang et al., 2008), and most of it is not reasonably utilized. Chinese wild rye grass hay has been extensively included in ruminant diets (Yang et al., 2009).

Contents of CP, NDF, and NFC are different among these forage sources. Compared with corn stover and Chinese wild rye grass, alfalfa hay is higher in contents of CP, RDP, and RUP (Zhao and Li, 2009). Alfalfa hay contains a higher NFC content, thus providing more energy available for the capture of RDP in the rumen compared with corn stover and Chinese wild rye grass. Increasing RUP by 1% can improve milk production by 1 kg (NRC, 2001). Providing both RDP and RUP may meet the requirement for MP, whereas the increasing MP supply can increase not only milk yield but also milk protein yield (Wright et al., 1998). In a previous study, a strong correlation was observed between dietary MP content and 4% FCM yield or milk protein yield (Wang et al., 2007).

It is a strategic policy for the Chinese dairy industry to make full use of crop residues such as corn stover to meet the demand of forage and reduce the dependence on imported alfalfa hay. However, no study has been conducted to compare the effects of substituting corn stover or Chinese wild rye grass for alfalfa hay as a forage source for dairy cows. Because of low CP and energy contents in corn stover and Chinese wild rye grass, protein and energy supplementation is needed to meet the requirements for lactation. Therefore, the objective

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of this study was to investigate the effect of these forage sources on the lactation performance, microbial protein (**MCP**) synthesis, and N utilization efficiency in early lactation dairy cows fed isonitrogenous and isocaloric diets.

MATERIALS AND METHODS

Animals and Experimental Design

The use of animals was approved by the Animal Care Committee of Zhejiang University (Hangzhou, China). Twelve primiparous Chinese Holstein cows (552 ± 16.0 kg BW; 45 ± 6.0 DIM) were separated into 3 groups and randomly allocated to 1 of 3 dietary treatments in a 3 × 3 Latin square design. Groups were balanced for DIM and milk yield.

The experimental diets (Table 1) had a forage-to-concentrate ratio of 45:55 (DM basis), with a similar component of concentrate but with different forage sources as follows: (1) TMR containing corn stover (**CS**), (2) TMR containing Chinese wild rye grass (**CWR**), or (3) TMR containing alfalfa hay (**AH**). All the diets were isonitrogenous and isocaloric and formulated to meet the NE_L requirements of cows for milk production of 30 kg/d (Ministry of Agriculture of P.R. China, 2004). Feed was offered ad libitum to allow for at least 5 to 10% orts.

Each experimental period consisted of 14 d for adaptation, followed by 7 d for sample collection. The cows were housed in a tiestall barn and fed and milked 3 times daily at 0600, 1330, and 2000 h. All of the cows had free access to drinking water throughout the experiment.

Sampling Collection and Measurements

The feed offered and refused was weighed for 3 consecutive days on d 17, 18, and 19 of each period to determine the DMI. Ort samples for each cow were collected in proportion to the wet weight for each day. The forages and concentrates were sampled weekly to determine the DM content. Daily feed and ort samples were pooled by treatment, period, and cow, and then the subsample was used for chemical analysis. Samples were dried in a forced-air oven at 60°C for 48 h and stored in sealed plastic containers at −20°C until analyses. Dried samples were ground through a 2-mm screen in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) and then through a 1-mm screen in a Cyclotec mill (Tecator 1093; Tecator AB, Höganäs, Sweden) before analysis. The contents of DM, OM, ash, NPN, soluble CP, and total N of the ingredients, orts, and feces were determined according to the procedures of the Association of Official Analytical Chemists (AOAC, 1990). Starch was detected using a colorimetric method (Bertrand et al., 2003). The NDF, lignin, NDIN, and ADIN were analyzed by the method of Van Soest et al. (1991). Diet compositions that were calculated according to the chemical analysis and inclusion rate of ingredients are presented in Table 2. At the beginning and end of each period, cows were weighed one by one on a weighing scale before morning feeding to calculate the change in BW.

Table 1. Ingredients of the experimental diets

	Treatment[1]		
Item (% of DM)	CS	CWR	AH
Corn silage	21.0	19.0	19.0
Chinese wild rye grass hay	0.00	21.0	9.00
Corn stover	19.0	0.00	0.00
Alfalfa hay	5.00	5.00	17.0
Ground corn grain	28.0	26.6	28.0
Soybean meal, 46.3% CP	13.0	12.0	10.0
Cottonseed meal	3.50	3.50	3.50
Wheat bran	0.00	3.00	4.00
DDGS[2]	6.00	6.00	6.00
Dicalcium phosphate	1.20	0.85	0.75
Limestone	0.75	0.80	0.50
Sodium bicarbonate	0.75	0.75	0.75
Salt	0.50	0.50	0.50
Premix[3]	1.00	1.00	1.00
Ca salts of long-chain FA	0.30	0.00	0.00

[1]CS = TMR containing corn stover as the main forage; CWR = TMR containing Chinese wild rye grass as the main forage; AH = TMR containing alfalfa hay as the main forage.

[2]Distillers dried grains with solubles.

[3]Formulated to provide (per kilogram of DM) 500,000 to 700,000 IU of vitamin A, 140,000 to 170,000 IU of vitamin D_3, 2,000 to 4,000 IU of vitamin E, 7,000 to 9,000 mg of Zn, 40 to 80 mg of Se, 180 mg of I, 1,400 to 2,500 mg of Fe, 15 to 30 mg of Co, 1,400 to 2,500 mg of Mn, and 1,400 to 2,500 mg of Cu.

Table 2. Chemical composition of the experimental diets[1]

	Treatment[2]		
Composition	CS	CWR	AH
CP (% of DM)	16.2	16.1	16.3
Soluble CP (% of CP)	38.8	38.7	39.4
NPN (% of SCP)	24.4	24.2	26.5
NDIN (% of CP)	11.3	12.9	13.8
ADIN (% of CP)	5.30	5.70	6.40
NDF (% of DM)	36.4	36.0	33.0
NFC[3] (% of DM)	35.2	35.9	38.7
Lignin (% of DM)	6.00	6.20	6.10
Sucrose (% of DM)	7.60	8.80	9.60
Starch (% of DM)	23.5	22.9	24.4
Ca[4] (% of DM)	0.85	0.76	0.78
P[4] (% of DM)	0.58	0.59	0.56
NE_L[4] (Mcal/kg of DM)	1.59	1.59	1.61

[1]Compositions of experimental diets were calculated according to the chemical analysis and inclusion rate of ingredients as indicated in Table 1. Chemical compositions of the main forages are as follows: corn stover (% of DM): 84.4% OM, 6.5% CP, 66.5% NDF, and 36.2% ADF; Chinese wild rye grass hay (% of DM): 88.6% OM, 7.7% CP, 67.5% NDF, and 35.2% ADF; and alfalfa hay (% of DM): 89.0% OM, 16.9% CP, 39.4% NDF, and 30.6% ADF.

[2]CS = TMR containing corn stover as the main forage; CWR = TMR containing Chinese wild rye grass as the main forage; AH = TMR containing alfalfa hay as the main forage.

[3]Calculated as 100 − (% NDF + % CP + % ether extract + % ash).

[4]Calculated based on Ministry of Agriculture of P.R. China (2004).

Cows were milked at 0630, 1430, and 2130 h daily. The milk production was recorded and milk samples were collected on d 17, 18, and 19 of each period using milk-sampling devices (Waikato Milking Systems NZ Ltd., Waikato, Hamilton, New Zealand). Two 50-mL aliquots of the milk samples were collected daily at each milking and pooled in a proportion of 4:3:3. One subsample with added Bromopol (milk preservative; D & F Control Systems Inc., San Ramon, CA) was stored at 4°C for later analysis of the protein, fat, and lactose by infrared analysis (Laporte and Paquin, 1999) using a spectrophotometer (Fossomatic 4000; Foss Electric A/S, Hillerød, Denmark). The subsample without Bromopol was stored at −20°C for the analysis of MUN, according to the diacetyl monoxime-binding method described by Wang et al. (2010).

Blood samples (10 mL) were collected from the coccygeal vein of each cow into heparinized test tubes at approximately 3 h after feeding on d 20 of each period. The sample was then centrifuged at 3,000 × g for 15 min to obtain the plasma. All of the plasma samples were stored at −20°C for the later analysis of BUN according to the procedure described by Wang et al. (2007).

In Situ Digestion in the Rumen

Three ruminally cannulated Chinese Holstein cows (604 ± 36 kg of BW; 15 ± 2.4 kg of milk yield; 213 ± 6.5 DIM) were housed in individual stalls for the determination of the in vitro rumen OM and CP degradation of all 3 diets. The ingredients (% of DM) of the diet for the cannulated cows included 48.5% concentrate, 16.9% corn silage, 27.7% AH, and 6.9% CWR. The diet was fed 3 times daily for a total intake of 1.5% of the BW. The samples for the degradation study were dried at 65°C and ground through a 3-mm screen in a mill (Arthur H. Thomas Co.). About 5 g of each sample was placed into a nylon bag (10 × 20 cm; 50-μm pore size; Ankom Technology Corp., Macedon, NY) in 6 duplicates. The bags were tied to the end of a 40-cm nylon line and attached to a stainless-steel weight, and then placed in the ventral sac of the rumen through ruminal cannula to incubate for 2, 4, 8, 12, 16, 24, 36, and 48 h. After removal from the rumen, the bags were rinsed thoroughly in cool running tap water until the wash water ran clear. The samples were dried at 65°C in an oven and weighed to determine the residue mass and the residues were then pooled by the duration within each cow. The residues and original diet samples were ground to pass through a 1-mm screen in a Cyclotec mill (Tecator 1093; Tecator AB) before analysis of the DM, OM, and CP. The in situ digestion constants were estimated using the following nonlinear model (Ørskov et al., 1980): $p = a + b[1 - \exp(-ct)]$, where p = the rate of disappearance at time t (h), a = the rapidly degradable fraction in the rumen, and b = the fraction slowly degraded at rate c ($c > 0$). The effective degradability (**dg**) was calculated by assuming a passage rate (***kp***) of 8%/h (Madsen and Hvelplund, 1985), using the

formula of Ørskov et al. (1980): dg = $a + bc/(c + kp)$, where a, b, c, and kp are the constants described above.

Estimation of MCP Yield and MP

Urinary purine derivatives were used to estimate the MCP yield in the rumen (Chen and Gomes, 1992). Spot urine samples during the lactation trial were collected twice daily at approximately 6 and 12 h after feeding on d 17, 18, and 19 of each period. The daily urine samples were pooled by cow and 20-mL subsamples were acidified immediately with 80 mL of 0.036 mol of H_2SO_4/L and stored at −20°C for later analysis. At the end of the trial, all of the urine samples were thawed at 25°C and filtered through Whatman no. 1 filter paper (Broderick et al., 2008). The filtrates were then analyzed for urea N using the colorimetric method (Rahmatullah and Boyde, 1980). The purine derivatives were analyzed by the procedure of Chen and Gomes (1992) and creatinine was analyzed using a picric acid assay (Oser, 1965). Creatinine has been validated as a marker to estimate urine volume (Leonardi et al., 2003) and was assumed to be excreted at a rate of 29 mg/kg of BW for calculating the urine volume excretion rate (Valadares et al., 1999).

The MP was estimated as the sum of the intestinally absorbable dietary protein (**IADP**) and intestinally absorbable MCP (**IAMCP**). The IADP was estimated by the equation: IADP = RUP × CP intake × IDP, where **IDP** is the intestinal digestibility of RUP, determined from the residue of feedstuff incubated in the rumen for 16 h, according to a modified 3-step procedure (Gargallo et al., 2006). The IAMCP was estimated by the equation: IAMCP = MCP × 0.64 (NRC, 2001).

Statistical Analysis

Statistical analyses were carried out using SAS software (SAS Institute, 2000). Data within each treatment group were checked for normality using PROC UNIVARIATE NORMAL. Intake of DM, milk yield and composition, milk efficiency, MCP yield, MUN, and urinary N were analyzed as a multiple Latin square using PROC MIXED with covariance type AR (1). For lactation performance, DMI, milk efficiency, MCP yield, MUN, and urinary N, the statistical model included square, period, treatment, day, and treatment × period interaction, with the square, treatment, day, and their interactions as the fixed effects and the cow within the treatment as the random effect. For BW gain and BUN, the statistical model included the square, period, treatment, and treatment × period interaction, with the square, treatment, and their interactions as the fixed effects and the cow within the treatment as the random effect. The square × treatment interactions were originally evaluated but were removed from the final statistical models because they were not significant for any of the variables. Results were reported as LSMEANS and the differences between the treatments were detected using the Tukey adjustment.

Data on the constants of the OM and CP degradation in the rumen (a, b, and c), dg, RUP, and intestinal digestion parameters were analyzed using PROC GLM of SAS (SAS Institute, 2000). For the regression analysis, the REG procedure was used to evaluate the MP and microbial protein yield relative to the milk protein yield. A statistically significant difference was defined at $P < 0.05$ and trends were declared at $0.05 \leq P \leq 0.10$.

RESULTS

Feed Intake, Milk Yield, and Composition

The milk yield and composition are presented in Table 3. Daily DMI did not differ ($P = 0.30$) among the treatments, with an average of 17.6 kg/d. The milk yield was higher for cows fed AH than those fed CS ($P = 0.07$), resulting in a higher milk efficiency (milk yield/DMI) for AH than for CS ($P = 0.01$) with an intermediate value for CWR. The milk protein content was also higher for the cows fed AH compared with those fed CWR ($P = 0.01$; 3.02 vs. 2.92%), with no difference between the CWR and CS values. The contents of milk fat ($P = 0.12$) and milk lactose ($P = 0.17$) did not show differences among the treatments. The BW gain was similar among the diets ($P = 0.45$).

In Situ Digestion in the Rumen

The results for the rumen degradation of the OM and CP are presented in Table 4. For the OM degradation, AH had higher a ($P = 0.01$), b ($P < 0.01$), and c values ($P < 0.01$); thus, the dg value of OM for AH was higher ($P = 0.01$) compared with CS and CWR (53.2 vs. 47.8 and 47.8%). The a and c values for CP degradation were similar among the treatments, whereas CS exhibited a lower ($P = 0.01$) b value than AH and CWR. The dg value of CP for AH was higher ($P = 0.01$) than that for CS, with no difference between CS and CWR. The RUP for AH was lower ($P = 0.04$) than that for CS, with no difference between CWR and AH or CS (Table 4).

MCP Yield and MP

The MCP yield for AH was 17.2% higher than that for CS ($P = 0.01$; Table 5) and 15.3% higher than for

Table 3. Effects of corn stover, Chinese wild rye grass, and alfalfa hay as dietary forage sources on the DMI and milk production

	Treatment[1]			
Item	CS	CWR	AH	SEM
DMI (kg/d)	17.8	17.6	17.3	0.27
Milk yield (kg/d)	25.1	25.8	26.9	0.59
Milk composition (%)				
Protein	2.95[ab]	2.92[b]	3.02[a]	0.031
Fat	3.54	3.57	3.66	0.046
Lactose	5.07	5.07	5.13	0.035
BW gain (g/d)	153	146	154	8.7
Milk efficiency[2]	1.41[b]	1.47[ab]	1.56[a]	0.037

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

[1]CS = TMR containing corn stover as the main forage; CWR = TMR containing Chinese wild rye grass as the main forage; AH = TMR containing alfalfa hay as the main forage.

[2]Milk efficiency = milk yield/DMI.

CWR ($P = 0.02$; Table 5). The N conversion (milk N/N intake) for AH was higher than that for CS and CWR ($P < 0.01$). Feeding AH resulted in a lower concentration of urea N in the blood ($P < 0.01$), urine ($P = 0.04$), and milk ($P < 0.01$) than CS or CWR (Table 5).

The IDP, IADP, IAMCP, and MP values are presented in Table 6. No effects of the treatments were observed on the IDP ($P = 0.21$) and IADP ($P = 0.55$). The IAMCP with AH was higher than that of CS ($P = 0.01$) and CWR ($P = 0.01$). Diet AH demonstrated 10.0 and 8.9% higher MP values than CS and CWR, respectively. A significant correlation existed of the milk protein yield to the MP (Figure 1A) or microbial protein yield (Figure 1B) in lactating cows.

DISCUSSION

Reports in the literature on the effect of forage source on the microbial protein synthesized in the rumen are inconsistent (Khorasani et al., 2001; Voelker Linton and Allen, 2009). From a summary of 28 experiments, Clark et al. (1992) concluded that the ruminal degradation of OM is the predominant factor contributing to the MCP yield. A meta-analysis of studies on the effect of the dietary carbohydrate content in dairy cow diets indicated that a greater dietary concentration of NFC increased the MCP yield (Nocek and Russell, 1988), whereas a lower availability of readily fermentable carbohydrates had a negative effect on the MCP

Table 4. The constants of OM and CP degradation based on the equation $p = a + b[1 - \exp(-ct)]$,[1] and their effective degradability (dg) and RUP of the experimental diets

	Treatment[2]			
Item	CS	CWR	AH	SEM
OM degradation				
a (%)	27.5[b]	27.1[b]	33.1[a]	0.96
b (%)	59.2[a]	60.4[a]	52.3[b]	0.99
c (%/h)	4.20[b]	4.16[b]	5.00[a]	0.077
dg[3]	47.8[b]	47.8[b]	53.2[a]	0.91
CP degradation				
a (%)	23.9	24.0	24.7	0.84
b (%)	60.0[b]	63.8[a]	64.0[a]	0.70
c (%/h)	6.47	6.41	6.67	0.095
dg[3]	50.7[b]	52.3[ab]	53.8[a]	0.70
RUP[4] (% of CP)	49.3[a]	47.7[ab]	46.2[b]	0.70

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

[1]p = the rate of disappearance at time t (h), a = the rapidly degradable fraction in the rumen, and b = the fraction slowly degraded at rate c ($c > 0$).

[2]CS = TMR containing corn stover as the main forage; CWR = TMR containing Chinese wild rye grass as the main forage; AH = TMR containing alfalfa hay as the main forage.

[3]dg = $a + bc/(c + kp)$ (Ørskov et al., 1980), assuming a passage rate (kp) of 8%/h (Madsen and Hvelplund, 1985). Dg = degradability.

[4]RUP = 100 − RDP.

yield (Stern and Hoover, 1979; Lascano and Heinrichs, 2011). Compared with CS and CWR, AH had higher

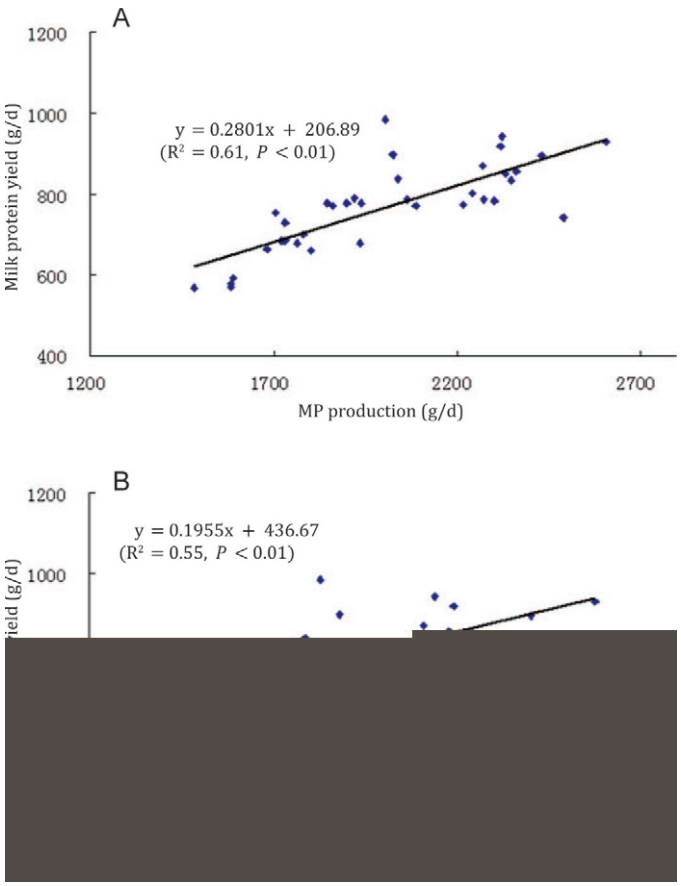

Figure 1. Regressions of the milk protein yield on the MP (A) and microbial protein (MCP) yield (B) in lactating cows. Color version available in the online PDF.

but the reduced BUN in the cows fed AH would benefit reproduction. A reduction in urinary N excretion was also observed when the MCP yield was increased (Broderick et al., 2008). Most of urinary N is excreted as urea, which can be rapidly hydrolyzed to ammonia and volatilized into the atmosphere (Broderick, 2003). Therefore, replacing CS or CWR with AH may help reduce the environmental burden of Chinese dairy production and improve animal health.

Another major source of urinary N is derived from the incomplete conversion of absorbed AA into productive uses such as milk protein and tissue production (Tamminga, 1992). In the current study, the urea in urine and milk with the CS diet was higher than with the AH diet, whereas the milk protein excretion was much lower. The results indicate that AH may have had a better AA balance and resulted in more efficient AA utilization for productive uses compared with CS and CWR. Additional work is needed to improve the utilization efficiency of feed resources available locally, such as CS or CWS, through the increased supply of rumen-fermentable energy and improved AA balance.

CONCLUSIONS

Compared with CS and CWR, the higher degradation of the rumen OM and the more readily fermentable carbohydrates for AH provided an increased energy supply for MCP synthesis in the rumen and the greater MP supply resulted in an increased yield of milk protein. The improved N conversion efficiency with decreased concentrations of urea N in the urine, plasma, and milk may benefit both animal health and environmental protection. We infer that a sufficient supply of the energy available for MCP synthesis is critical for lactating dairy cows fed forage of low quality, such as CS and CWR.

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