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Hydroponic barley supplementation fed with high-protein diets improves the production performance of lactating dairy cows

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ABSTRACT

The study investigated the effects of dietary protein level and the inclusion of hydroponic barley sprouts (HB) on lactation performance, blood biochemistry and N use efficiency in mid-lactation dairy cows. Treatments were arranged in a 2 × 2 factorial design with 2 CP levels (16.8% and 15.5% of DM), with HB (4.8% of DM, replacing 4.3% of alfalfa hay and 0.5% of distillers dried grains with solubles [DDGS]) or without HB. Forty-eight multiparous Holstein dairy cows (146 ± 15 DIM, 40 ± 5 kg/d of milk) were randomly allocated to 1 of 4 diets: high-protein diet (16.8% CP, HP), HP diet with HB (HP+HB), low-protein diet (15.5% CP, LP), or LP diet with HB (LP+HB). An interaction between CP × HB on DMI was detected, with DMI being unaffected by HB inclusion in cows fed the high-protein diets, but was lower in cows fed HB when the low-protein diet was fed. A CP × HB interaction was also observed on milk and milk protein yield, which was higher in cows fed HB with HP, but not LP. Inclusion of HB also tended to reduce milk fat content, and feeding HP resulted in a higher milk protein and MUN content, but lower milk lactose content. Feed efficiency was increased by feeding HP or HB diets, whereas N use efficiency was higher for cows fed LP or HB diets. There was an interaction on the apparent total-tract digestibility of DM and CP, which was higher when HB was fed along with HP, but reduced when fed with LP, whereas the digestibility of ADF was increased by feeding low-protein diets. In conclusion, feeding a low-protein diet had no adverse effect on cow performance, while feeding HB improved milk and milk component yield, and N efficiency when fed with a high-CP diet, but compromised cow performance with a low-CP diet.

Key words: dairy cow, dietary protein, feed efficiency, hydroponic barley, nitrogen utilization

INTRODUCTION

Dietary protein concentration is positively associated with DMI and production performance of dairy cows (Law et al., 2009). In pursuit of higher milk yield, high-protein diets are widely used in dairy production. However, when the dietary protein content exceeds the requirement for milk production this often results in more N excretion in the feces and urine, which increases the risk of environmental pollution (Olmos Colmenero and Broderick, 2006) and, due to the higher cost of protein feeds, raises dietary costs. Lowering the dietary protein level can also contribute to an improvement in N use efficiency (**NUE**) for milk production (Law et al., 2009; Kidane et al., 2018; Chowdhury et al., 2023). However, reducing dietary N supply may also reduce DMI and milk production (Hristov et al., 2004; Sinclair et al., 2014). To counteract these possible adverse effects, numerous strategies have been developed. Supplementation with rumen-protected AA to increase the supply of limiting EAA to the small intestine is one of the most common ways (Lee et al., 2012), while maximizing microbial protein synthesis by optimizing rumen fermentation can also increase metabolizable protein supply to the small intestine (Sinclair et al., 2014).

Hydroponic barley sprouts (**HB**) is a fresh forage that can be germinated and grown under optimized temperature, humidity, light and gas environment for a short period of time in special chambers, producing palatable sprouts with a height of 15 to 20 cm that can be provided without the limitation of climate and arable land (Farghaly et al., 2019; Ali et al., 2021). There are several changes that occur to the chemical composition

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

of barley during the process of sprouting. As the results of increasing enzyme levels, proteins are degraded into peptides and converted to AA, whereas carbohydrates are transformed to sugar, and fats are converted to fatty acids (Mohsen et al., 2015). The phenolic content of barley grain is also increased by sprouting, thereby enhancing its antioxidant activity (Nemzer et al., 2019; Niroula et al., 2019). Meanwhile, the concentration of CP and vitamins in HB are increased compared with barley grain, and the availability of certain minerals are also increased, with the phytate in seeds degraded by phytases (Fazaeli et al., 2012; Abouelezz et al., 2019). With improved nutrient availability and digestibility, the anticipated benefits of HB on ruminant performance have been demonstrated in some studies. For example, Hafla et al. (2014) compared the effect of HB and barley grain on nutrient digestibility with a continuous-culture fermentor system, and reported that the true DM digestibility of the diet was increased from 76.0% to 79.0% when barley grain (7% of dietary inclusion) was replaced by HB in the diet. Farghaly et al. (2019) evaluated the effect of HB on rumen fermentation and ruminal enzyme activity of sheep, and found that supplementing sheep with HB alone or HB in combination with a concentrate mixture (meeting 60% of their nutritional requirements), increased the total VFA concentration by 9.6%, the relative proportion of propionate by 9.0%, and rumen urease activity by 37.5%, when compared with sheep supplemented with only Egyptian clover or Egyptian clover with a concentrate mixture. Raeisi et al. (2018) also replaced barley grain with HB in the diet of sheep, and reported that N retention increased linearly with HB dosage, with N retention in the 21% HB group being 28.8 g/d, some 106% higher than that in the control group. Positive effects of HB on rumen fermentation and N metabolism may therefore compensate for any adverse effects of low-protein diets on animal performance. However, the combination of low CP and HB has not been studied in ruminants. We hypothesize that the dietary inclusion of HB would increase DMI, feed and N efficiency, and milk production in cows fed low-protein diets. The objective of this study was to determine the effect of dietary protein level, sprouted barley inclusion, and their interaction on the performance and metabolism of dairy cows.

MATERIALS AND METHODS

Animals and Diets

This study was approved by the Animal Care and Use Committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (No. IAS20180115). The use of animals in our study was in strict accordance with the Directions for Caring for Experimental Animals from the Institute of Animal Science, Chinese Academy of Agricultural Sciences. A statistical power analysis was carried out with an $\alpha = 0.05$ and power = 0.80, and the total sample size required with 0.4 effect size was 44 cows, as estimated using G power 3.1 software (Faul et al., 2009). Milk yield and DMI were the variables used to determine the sample size. An effect size (f) of 0.4 was used with variance explained by effect = 1.0, the variance within group = 5.25, and partial $\eta^2 = 0.15$. Consequently, a total of 48 cows were used in our study. Forty-eight second-parity mid-lactation Holstein cows (mean ± SD: 146 ± 15 DIM, 39.9 ± 4.87 kg/d of milk) were randomly assigned to 1 of 4 treatments (n = 12 per treatment) in a 2 × 2 factorial design with 2 CP levels (16.8% and 15.5% of DM), with HB (4.8% of DM, replacing 4.3% of alfalfa hay and 0.5% of distillers dried grains with solubles [**DDGS**]) or without HB. Cows were housed in a freestall pen containing sand-bedded stalls with access to individual feed bins (n = 48) equipped with automatic feeding gates (American Calan Inc., Northwood, NH). Each cow was fitted with a transponder attached to a collar situated around her neck that allowed access to a specific feed bin. Before being trained to use the feeding system, all the gates of the feed bins were kept open, and all cows were provided with the same diet for a period of 2 wk. This allowed the cows to become accustomed to using the feed bins, and their relative positions were monitored during this time. Afterward, the cows were trained to use the feeding system 1 wk before the start of the study. Cows remained on the study for 8 wk and received 1 of the 4 diets: high-protein diet (16.8% CP, **HP**), HP diet with hydroponic barley (**HP+HB**), low-protein diet (15.5% CP, **LP**), and LP diet with hydroponic barley (**LP+HB**). The 4 diets were balanced for NE_L, MP, minerals, and vitamins according to NRC (2001). The ingredient and chemical composition of the diets are presented in Table 1. The TMR was fed twice a day to ensure between 5% and 10% refusals, with refusals removed and weighed daily. Hydroponic barley was provided by Shandong Jiyuan Pastoral Technology Development Co. Ltd. China and grown in containers, with water and light supplied artificially, without the addition of any other nutrients. Briefly, barley seeds were evenly spread on a planting tray with a thickness of approximately 1 cm. The container temperature was set to 28°C, and the relative humidity was maintained at 80% during the seed germination phase, which lasted for 3 d in darkness. Throughout the growth period of the barley sprouts, the temperature in the container was adjusted to 26°C, and the relative humidity was controlled between 60% and 80%. To maintain the humidity in the containers, the water spraying cycle was set to 90 min, with each episode lasting 16 s, and ventilation was initiated when the humidity exceeded 80%. High-power red and blue light-emitting diode light sources were used for illumi-

Table 1. The ingredient and chemical composition of TMR fed to the cows during the trial

	Treatment[1]			
Item	HP	HP+HB	LP	LP+HB
Ingredient composition, % of DM				
Corn silage	29.9	29.9	29.9	29.9
High moisture corn	17.0	17.0	17.0	17.0
Alfalfa hay	11.6	7.3	11.6	7.3
Flaked corn	7.0	7.0	7.0	7.0
Soybean meal	6.8	6.8	6.8	6.8
Beet pulp	4.4	4.4	6.3	6.3
Cottonseed meal	4.3	6.1	4.3	6.1
Whole cottonseed	6.0	6.0	6.0	6.0
Barley grass	—	4.8	—	4.8
Premix[2]	4.5	4.5	4.5	4.5
DDGS	3.9	1.6	3.9	1.6
Corn gluten meal	1.9	1.9	—	—
Beer grains	1.6	1.6	1.6	1.6
Spouting corn bran	1.1	1.1	1.1	1.1
Chemical composition[3]				
DM, %	49.9	45.3	50.4	44.7
CP, % of DM	16.8	16.8	15.5	15.5
NDF, % of DM	33.8	34.8	35.6	34.4
ADF, % of DM	21.1	20.8	21.3	20.6
Starch, % of DM	24.6	24.8	24.8	24.7
NFC, % of DM	41.2	42.3	42.5	42.9
NE_L, Mcal/kg DM	1.65	1.67	1.64	1.66
RDP, % of DM	9.81	9.81	9.47	9.46
RUP, % of DM	6.99	6.99	6.03	6.04
MP, % of DM	11.68	11.70	11.05	11.09
Lys, % of MP	6.07	6.05	6.34	6.32
Met, % of MP	2.16	2.14	2.14	2.12
His, % of MP	2.61	2.60	2.64	2.64
Lys:Met	2.82	2.84	2.96	2.98

[1]HP: high dietary protein (CP = 16.8% of DM) without hydroponic barley; HP+HB: high dietary protein (HP, CP = 16.8% of DM) with hydroponic barley (HB, HB = 4.8% of DM); LP: low dietary protein (CP = 15.5% of DM) without hydroponic barley; LP+HB: low dietary protein (CP = 15.5% of DM) with hydroponic barley (HB, HB = 4.8% of DM).

[2]Premix: lactation premix contained (per kg of premix, DM basis): 100,000 IU of vitamin A, 28,000 IU of vitamin D_3, 1,000 IU of vitamin E, 100 mg of vitamin PP, 325 mg of Cu, 480 mg of Mn, 1,285 mg of Zn, 14 mg of I, 15 mg of Se, 11 mg of Co.

[3]NFC, NE_L, RDP, RUP, MP, Lys, Met, His, and Lys:Met were predicted based on NRC (2001).

nation, with a ratio of 7:3. The working cycle of light-emitting diode was 40 min, with the on time lasting 28 min. After 7 d of growth, the HB was harvested and fed fresh daily as part of the TMR. The chemical composition of the HB is shown in Table 2. Individual daily DMI was calculated by subtracting the refusals from the feed offered. Body condition score was determined by 3 independent observers using a 5-point scale (1 = thin to 5 = obese, with quarter-point increments; Roche et al., 2009; Velásquez et al., 2018) and fecal score was examined by 3 independent observers according to Hughes (2001) at wk 0, 4, and 8 of the study.

Sample Collection and Measurements

Cows were milked 3 times daily at 0600, 1400, and 2200 h. Individual milk yield was recorded at each milking. Weekly milk samples from each individual cow were collected on the final day of the week, over 3 consecutive milkings. The samples were then mixed based on the average milk production at each milking. Milk samples

Table 2. Chemical composition of the hydroponic barley(mean ± SD)[1]

Item, % of DM, unless otherwise stated	Hydroponic barley
DM, % of as fed	10.2 ± 2.17
CP	15.0 ± 0.06
Starch	10.5 ± 0.23
Ether extract	3.1 ± 0.04
NDF	47.4 ± 1.32
ADF	20.4 ± 0.06
Ash	2.6 ± 0.04
ADL	3.0 ± 0.03
NDIN	2.5 ± 0.12
ADIN	0.7 ± 0.01
NPN	8.9 ± 0.67

[1]Hydroponic barley was grown in containers, and water and light were supplied artificially, without adding other nutrients during growing. After 7 d growing, it was harvested for analysis and fed to dairy cows.

were preserved with bronopol-B2 at 4°C for subsequent analysis of fat, protein, lactose, and MUN, using a near-infrared reflectance spectroscopy analyzer (Foss Electric, Hillerød, Denmark). The 4% FCM yield was calculated as: 4% FCM = 0.4 × milk yield (kg/d) + 15 × milk fat yield (kg/d) (Palmquist and Conrad, 1978). Feed efficiency (**FE**) was calculated by dividing 4% FCM yield (kg/d) by DMI (kg/d). Energy-corrected milk yield was calculated using the following equation: ECM = [12.82 × milk fat yield (kg/d)] + [7.13 × milk protein yield (kg/d)] + [0.323 × milk yield (kg/d)] (Tyrrell and Reid, 1965). Apparent NUE was calculated as NUE = milk protein N (g)/N intake (g) × 100.

Total mixed rations were sampled for 3 consecutive days each week. Each type of TMR was sampled from 12 locations (each feed bin) at the morning feeding before cow access. The samples were homogenized by treatment and quartered to provide a 500 g sample every week for subsequent analysis. Hydroponic barley sprouts were collected at 5 locations per tray and 3 to 5 trays per day for 3 consecutive days each week. Collected HB samples were homogenized, and a 500-g subsample was obtained for later analysis. An in vivo digestibility trial was conducted during the final 3 d of the experiment according to the method of Zhong et al. (2008). In brief, fecal samples (300–500 g) were collected every 6 h from the rectum of each cow at 0000, 0600, 1200, and 1800 h on d 1; at 0200, 0800, 1400, and 2000 h on d 2; and at 0400, 1000, 1600, and 2200 on d 3, and feed samples were sampled daily during the in vivo digestibility trial. The fecal samples were pooled by cow and subsampled. All samples were dried at 65°C for 48 h in a forced-air oven, ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas Co., Philadelphia, PA), and stored at −20°C for nutrient analysis. Hydroponic barley sample was analyzed for DM (method 934.01), CP (method 976.06), ash (method 942.05), and ether extract (method 920.39) according to AOAC International (1995), starch using the method reported by Hall (2009), NDF and ADF by the method of Van Soest et al. (1991), NPN content as per Licitra et al. (1996), and NDIN, ADIN, ADL as described by Van Soest et al., (1991). Feed samples were analyzed for DM, CP, NDF, and starch, with RDP, NFC, and NE_L calculated according to the NRC (2001). Fecal sample was measured for DM, CP, NDF, and ADF content. The acid-insoluble ash (**AIA**) content in feed and fecal samples was determined by the method of Van Keulen and Young (1977) and used for the calculation of apparent total-tract digestibility (**ATTD**). The ATTD of the nutrient of each cow was calculated as follow: ATTD = [1 − (Nf × Ad)/(Nd × Af)] × 1,000 g/kg, where Nd and Nf were nutrient content in the diet and feces, respectively, and Ad and Af were AIA content in diet and feces, respectively.

Blood samples from each cow were collected from the coccygeal vein with an untreated vacutainer before morning feeding on d 28 and d 56 of the study. All blood samples were centrifuged immediately at 3,000 × *g* at 4°C for 15 min to harvest serum, which was separated into 4 aliquots and stored at −20°C until further analysis. Serum alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), total protein (**TP**), albumin (**ALB**), alkaline phosphatase (**ALP**), creatinine (**CR**), and BUN content were analyzed on an automated biochemistry analyzer (Hitachi 7600; Hitachi Co. Ltd., Tokyo, Japan) with kits from BioSino Bio-Technology and Science Inc. (Beijing, China) and Sekisui Medical (China) Co. Ltd. (Beijing, China). The inter- and intra-assay CV for ALT, AST, TP, ALB, ALP, CR, and BUN were 3.31% and 5.05%, 1.92% and 2.27%, 1.91% and 2.68%, 2.63% and 3.29%, 3.93% and 5.39%, 1.27% and 2.76%, and 3.71% and 9.30%, respectively. The total antioxidant capacity (**T-AOC**), malondialdehyde (**MDA**), superoxide dismutase (**SOD**), and glutathione peroxidase (**GSH-Px**) concentrations in serum were determined with a UV spectrophotometer (Yipu Co. Ltd., Shanghai, China), using kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The inter- and intra-assay CV for T-AOC, MDA, SOD, and GSH-Px were 1.77% and 4.99%, 1.91% and 8.57%, 1.18% and 2.00%, and 1.43% and 2.13%, respectively. The difference between TP and ALB was calculated as the serum globulin content.

Statistical Analysis

Data were analyzed as a completely randomized design with repeated measurements using the PROC MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). The MIXED statistical model used for analysis was as follows:

$$Y_{ijkl} = \mu + P_i + B_j + PB_{ij} + A_{ijk} + T_l + TP_{il} + TB_{jl} + \varepsilon_{ijkl},$$

where Y_{ijkl} was the dependent variable, μ was the overall mean, P_i was the fixed effect of CP (CP = 15.5% or 16.8% of DM), B_j was the fixed effect of hydroponic barley (j = without or with HB at 4.8% of DM); PB_{ij} was the interaction of CP and hydroponic barley; A_{ijk} was the random effect of the kth cow in the ijth combination of CP and HB; T_l was the fixed effect of time (i.e., wk); the 2-way interactions of time with TP_{il} (the interaction of CP and time) and TB_{jl} (the interaction of HB and time) were considered fixed, and ε_{ijkl} was the residual error. The Fisher's protected least significant difference (LSD) test was used for multiple treatment comparisons using the LSMEANS of SAS with letter grouping obtained using SAS pdmix800 macro (Saxton, 1998). Statistical

Table 3. Effect of protein level and hydroponic barley on productive performance of lactating dairy cows

Item	Treatment[1]				SEM	*P*-value		
	HP	HP+HB	LP	LP+HB		CP[2]	HB[3]	CP × HB
DMI, kg/d	24.8[b]	24.3[b]	26.4[a]	23.9[c]	0.15	<0.01	<0.01	<0.01
NE_L intake,[4] Mcal/d	40.8[b]	40.7[b]	43.2[a]	39.7[c]	0.25	<0.01	<0.01	<0.01
Milk and milk component yield								
Milk yield, kg/d	35.5[b]	38.3[a]	37.0[ab]	36.4[b]	0.59	0.72	0.08	<0.01
4% FCM yield,[5] kg/d	36.0[b]	37.8[a]	38.3[a]	36.1[b]	0.27	0.26	0.47	<0.01
ECM yield,[6] kg/d	39.2[b]	41.7[a]	41.5[a]	39.4[b]	0.28	0.95	0.50	<0.01
Milk fat yield, kg/d	1.45[ab]	1.49[ab]	1.54[a]	1.44[b]	0.034	0.59	0.33	0.04
Milk protein yield, kg/d	1.28[b]	1.41[a]	1.33[b]	1.29[b]	0.022	0.13	0.02	<0.01
Milk lactose yield, kg/d	1.78[b]	1.92[a]	1.91[a]	1.83[ab]	0.032	0.41	0.38	<0.01
Milk composition								
Fat, %	4.12	3.88	4.09	4.01	0.085	0.57	0.06	0.36
Protein, %	3.60	3.67	3.53	3.57	0.036	0.02	0.12	0.57
Lactose, %	4.99	4.98	5.06	5.01	0.025	0.03	0.30	0.51
TS, %	14.8	14.6	14.8	14.7	0.17	0.70	0.26	0.75
MUN, mg/dL	11.7	11.6	11.1	10.7	0.27	<0.01	0.39	0.52
FE[7]	1.50	1.58	1.46	1.52	0.013	<0.01	<0.01	0.64
NUE,[8] %	33.3	36.6	34.8	37.2	0.31	<0.01	<0.01	0.15
BCS	2.92	2.91	2.96	2.93	0.038	0.41	0.64	0.79
Fecal score	2.59[ab]	2.45[b]	2.36[b]	2.71[a]	0.089	0.90	0.26	<0.01

[a–c]Means from interactions in the same row with different superscripts are significantly different ($P < 0.05$) using the LSD method.

[1]HP: high dietary protein (CP = 16.8% of DM) without hydroponic barley; HP+HB: high dietary protein (HP, CP = 16.8% of DM) with hydroponic barley (HB, HB = 4.8% of DM); LP: low dietary protein (CP = 15.5% of DM) without hydroponic barley; LP+HB: low dietary protein (CP = 15.5% of DM) with hydroponic barley (HB, HB = 4.8% of DM). Twelve cows were in each group.

[2]CP (protein level): high-protein diet (CP = 16.8% of DM), low-protein diet (CP = 15.5% of DM).

[3]HB (hydroponic barley level): with or without hydroponic barley (4.8% of DM).

[4]NE_L was calculated based on NRC (2001).

[5]4% FCM yield calculated as FCM yield = 0.4 × milk yield (kg/d) + 15 × milk fat yield (kg/d).

[6]ECM yield calculated as ECM yield = [12.82 × milk fat yield (kg/d)] + [7.13 × milk protein yield (kg/d)] + [0.323 × milk yield (kg/d)].

[7]Feed efficiency calculated as FE = 4% FCM yield (kg/d)/DMI (kg/d).

[8]NUE, apparent N use efficiency, calculated as NUE = milk protein N (g)/N intake × 100%.

differences were declared significant at $P < 0.05$, and tendencies were stated at $0.05 \le P < 0.10$.

RESULTS

DMI and Milk Production Performance

The HB had a low DM content (10.2%) and contained 15.0% CP, 10.5% starch, 47.4% NDF, 20.4% ADF, and 2.6% ash (Table 2), and was considered to be a high-quality forage. The HB diets were formulated by replacing 4.3% of the alfalfa hay and 0.5% of DDGS. To ensure that both LP and HP diets were isonitrogenous, 1.8% of DDGS was replaced with cottonseed meal. The formulation of the low-protein diets was achieved by increasing 1.9% of beet pulp to replace all the corn gluten meal in the diet (Table 1). The chemical composition of our TMR is consistent with the predicted value obtained by NRC (2001).

There was an interaction between dietary protein level and HB inclusion on DMI and NE_L intake ($P < 0.05$; Table 3), with HB having no effect when cows were fed the high-protein diets, but decreased intake when fed the low protein level. There was also a CP × HB interaction ($P < 0.05$) in our study for milk, 4% FCM, and ECM yield, with HB increasing yield when fed with high-protein diets, but decreasing yield when fed with the low-protein diets. Additionally, the 4% FCM and ECM yield of cows fed HP+HB did not differ from LP, but was greater compared with cows fed the HP or LP+HB diets. There was also an interaction between CP and HB on milk component yield ($P < 0.05$), with HB increasing milk protein and lactose yield when fed with HP but not LP diets, and decreasing milk fat yield when fed with the LP diet. The inclusion of HB tended to reduce milk fat content ($P = 0.06$), and feeding high-protein diets resulted in a higher milk protein ($P < 0.05$) but lower milk lactose content ($P < 0.05$). In addition, the concentration of MUN was reduced ($P < 0.05$) when the dietary CP was decreased, with a mean concentration of 11.7 mg/dL in cows fed the high-protein diets, and 10.9 mg/dL in those fed the low-protein diets.

Table 4. Effect of protein level and hydroponic barley on blood biochemical parameters of lactating dairy cows

	Treatment[1]					*P*-value		
Item	HP	HP+HB	LP	LP+HB	SEM	CP[2]	HB[3]	CP × HB
Alanine aminotransferase, U/L	23.9	23.9	25.2	26.9	0.81	0.02	0.31	0.30
Aspartate aminotransferase, U/L	97.5	94.4	84.5	95.4	9.38	0.53	0.68	0.46
Total protein, g/L	72.3	74.4	74.5	74.3	2.06	0.62	0.65	0.60
Albumin, g/L	32.4	32.0	31.3	32.9	1.45	0.93	0.70	0.49
Globulin, g/L	39.9	42.4	43.2	41.5	3.23	0.72	0.91	0.52
Albumin:globulin	0.87	0.78	0.75	0.87	0.078	0.87	0.84	0.19
Alkaline phosphatase, U/L	38.9	51.8	36.2	37.4	4.680	0.08	0.14	0.22
Creatinine, μmol/L	63.6	71.4	69.0	69.1	2.84	0.58	0.17	0.18
BUN, mmol/L	5.14	5.26	4.52	4.57	0.230	<0.01	0.71	0.87
Total antioxidant capacity, U/mL	2.17	2.21	2.23	2.26	0.105	0.63	0.73	0.96
Malondialdehyde, nmol/mL	6.72	6.60	6.03	6.17	0.308	0.08	0.98	0.67
Superoxide dismutase, U/mL	158.3	159.4	156.3	151.0	6.519	0.43	0.75	0.63
Glutathione peroxidase, U/mL	99.9	98.3	103.3	100.5	6.00	0.64	0.72	0.92

[a,b]Means from interactions in the same row with different superscripts are significantly different ($P < 0.05$) using the LSD method.

[1]HP: high dietary protein (CP = 16.8% of DM) without hydroponic barley; HP+HB: high dietary protein (HP, CP = 16.8% of DM) with hydroponic barley (HB, HB = 4.8% of DM); LP: low dietary protein (CP = 15.5% of DM) without hydroponic barley; LP+HB: low dietary protein (CP = 15.5% of DM) with hydroponic barley (HB, HB = 4.8% of DM). Twelve cows were in each group.

[2]CP (protein level): high-protein diet (CP = 16.8% of DM); low-protein diet (CP = 15.5% of DM).

[3]HB (hydroponic barley level): with or without hydroponic barley (4.8% of DM).

Effects on FE, NUE, BCS, and Fecal Score

There were no CP × HB interactions for FE and NUE, but there were main effects of both CP ($P < 0.05$) and HB ($P < 0.05$; Table 3). Feeding the high- compared the low-protein diets increased FE ($P < 0.05$) but decreased NUE ($P < 0.05$), while the inclusion of HB increased both FE and NUE ($P < 0.05$). We observed a CP × HB interaction on fecal score ($P < 0.05$), which was highest in cows fed LP+HB, and was higher than HP+HB and LP, but not HP.

Effects on Blood Biochemical Parameters

We found no CP × HB interactions, or effects of HB on blood biochemical parameters in our study ($P > 0.05$; Table 4). Serum ALT concentration was increased in cows fed the low compared with the high-protein diets ($P < 0.05$), while ALP concentration tended ($P = 0.08$) to be higher in cows fed the high- compared with the low-protein diets. Serum BUN concentration was decreased ($P < 0.05$), and serum MDA concentration tended to be decreased ($P = 0.08$) by feeding the low dietary CP level.

Effects on Nutrient Intake and ATTD

There was an interaction ($P < 0.05$) between CP and HB on the intake of N, RDP, NDF, and ADF (Table 5). Specifically, cows on HP diet exhibited the highest N intake, followed by those on HP+HB and LP diets, with the lowest intake observed in cows fed LP+HB. The intake of RDP or ADF was highest in cows fed LP, followed by HP and HP+HB, with those offered LP+HB having the lowest intake. Regarding NDF intake, cows fed LP had the highest intake, followed by those on HP and HP+HB, which did not differ, with the lowest intake in cows fed LP+HB. There was also an interaction between CP and HB on the predicted ruminal MP outflow ($P < 0.05$), with cows receiving the LP or HP diets having the highest predicted ruminal MP outflow, and those fed the LP+HB diet the lowest.

There was a CP × HB interaction ($P < 0.05$) on the ATTD of DM (**DMD**) and CP (**CPD**), with DMD being highest in cows fed LP ($P < 0.05$), intermediate in HP+HB cows, and lowest for those fed HP or LP+HB. The CPD was also highest in LP cows ($P < 0.05$), intermediate in HP+HB cows, and lowest in HP ($P < 0.05$), but did not differ between cows fed LP+HB and either HP containing diets. There was no effect of treatment on the ATTD of NDF, with a mean value of 49.5% of nutrient intake. However, the main effect of CP level on ADF digestibility, was that nutrient intake was 3.45 percentage points higher in dairy cows fed the low CP level ($P < 0.05$).

DISCUSSION

Dry Matter Intake

Generally, dietary DMI is increased with dietary CP level, which may partly be due to the positive effect of ration CP content on fiber and DM digestibility (NASEM, 2021), and consequently a reduction in rumen distention. In a meta-analysis, Zanton (2016) reported a positive relationship between dietary CP content and DMI in both continuous and change-over experimental

Table 5. Effect of protein level and hydroponic barley on nutrient intake and apparent total-tract digestibility of nutrients for lactating dairy cows

	Treatment[1]					*P*-value		
Item	HP	HP+HB	LP	LP+HB	SEM	CP[2]	HB[3]	CP × HB
Nutrient intake, g/d								
Nitrogen intake	665[a]	655[b]	654[b]	594[c]	3.9	<0.01	<0.01	<0.01
Predicted RDP intake	2,428[b]	2,389[c]	2,494[a]	2,267[d]	14.4	0.05	<0.01	<0.01
NDF intake	8,367[bc]	8,472[b]	9,383[a]	8,235[c]	51.5	<0.01	<0.01	<0.01
ADF intake	5,223[b]	5,064[c]	5,614[a]	4,931[d]	31.3	<0.01	<0.01	<0.01
Predicted ruminal outflow of MP	2,891[ab]	2,849[b]	2,939[a]	2,679[c]	17.1	<0.01	<0.01	<0.01
ATTD of nutrients, % of nutrient intake								
DMD[4]	67.0[c]	69.7[b]	71.6[a]	67.8[c]	0.59	0.03	0.39	<0.01
CPD[5]	70.3[c]	73.3[b]	75.6[a]	71.7[bc]	0.71	0.02	0.59	<0.01
NDF digestibility[6]	49.9	49.5	50.8	47.6	2.19	0.83	0.42	0.52
ADF digestibility[7]	43.2	44.7	46.8	48.0	1.60	0.04	0.42	0.90

[a–d]Means from interactions in the same row with different superscripts are significantly different ($P < 0.05$) using the LSD method.

[1]HP: high dietary protein (CP = 16.8% of DM) without hydroponic barley; HP+HB: high dietary protein (HP, CP = 16.8% of DM) with hydroponic barley (HB, HB = 4.8% of DM); LP: low dietary protein (CP = 15.5% of DM) without hydroponic barley; LP+HB: low dietary protein (CP = 15.5% of DM) with hydroponic barley (HB, HB = 4.8% of DM). Twelve cows were in each group.

[2]CP (protein level): high-protein diet (CP = 16.8% of DM), low-protein diet (CP = 15.5% of DM).

[3]HB (hydroponic barley level): with or without hydroponic barley (4.8% of DM).

[4]DMD: apparent total-tract digestibility of DM.

[5]CPD: apparent total-tract digestibility of CP.

[6]Apparent total-tract digestibility of NDF.

[7]Apparent total-tract digestibility of ADF.

designs. Additionally, given the conversion of starch in grains to simple sugars and the activation of certain enzymes in sprouts, an improvement in palatability and voluntary intake was anticipated with hydroponic barley feeding. Raeisi et al. (2018) corroborated this, reporting a linear increase in DMI when sheep were fed HB at 7%, 14%, 21% of DM. Therefore, it was hypothesized that HB feeding would counteract the detrimental effects of a low-protein diet on DMI. However, contrary to expectations, cows on LP+HB diet exhibited the lowest DMI in our study. This may be due to high water content of HB, which reduced the DM content of the TMR (50.4% vs. 44.7% for no and HB inclusion, respectively), increased rumen fill, and consequently led to a decrease in DMI (Felton and DeVries, 2010; Fazaeli et al., 2021). Supporting this result, Morales et al. (2009) reported a linear decrease in DMI when rabbits were fed HB at rates of 10%, 20%, and 30% of their commercial feed. It is important to note that the inclusion of HB also led to a decrease in the DM content of HP+HB diet, from 49.9% to 45.3%. However, this reduction did not result in a lowered DMI. It is possible that the lower ruminal N supply in the LP+HB diet compared with the HP+HB diet, might have compromised ruminal fiber digestion, potentially decreasing both the passage rate and DMI (NASEM, 2021).

In our study, the low-protein diets were predicted to provide a lower concentration of RDP and MP, as well as a lower levels of dietary Lys, Met, and His, despite a higher Lys percentage relative to MP in these diets. However, defying our initial predictions, cows fed the LP diet exhibited the highest DMI. The result is consistent with Komaragiri and Erdman (1997), who reported that cows fed high-protein diet consumed less DM for the first 7 wk of postpartum, and attributed this result to the high content of animal by-products in the high-protein diet, which leads to poor palatability. We formulated the high-protein diets by replacing a proportion of the beet pulp with corn gluten meal, which is unlikely to have affected palatability, and there is no clear reason why intake was higher in the LP diet in our study.

Milk Production and Composition

Feeding HB tended to increase milk yield in our study, particularly in cows fed the high-protein diet. Farghaly et al. (2019) reported that HB increased the concentration of total VFA and propionate in the rumen, and speculated that this may be due to an increased supply of vitamins and enzyme, which act as bioactive catalysts to promote the metabolism of feed and increase the release of energy with the high N concentration in rumen, resulting in a greater ruminal nutrient outflow, leading to a higher milk yield. Nevertheless, the expected enhancement of milk production via an HB-mediated improvement in ruminal fermentation was not discernible in cows fed the low-protein diet in our study. This may primarily be ascribed to the lower DMI and intake of NDF and ADF in cows fed LP+HB, which likely restricted the ruminal supply of energy. Furthermore, despite an anticipated improvement in rumen fermentation, the lower consumption of N and RDP could have limited microbial growth in the rumen, thereby contributing to the lower predicted ruminal outflow of MP and subsequent effect milk yield of cows on the LP+HB diet. The higher DMI, NE_L intake, DMD, and CPD observed in the LP group could have supported an increased milk yield, ECM, 4% FCM, milk protein, and lactose production at levels equivalent to or surpassing those in HP diet.

Milk fat and protein contents are important economic parameters, and in our current study, milk fat content tended to be decreased by feeding HB, which may be associated with the higher milk yield in cows fed HB, as milk fat content is often negatively correlated with milk yield (Yoon et al., 2004). Additionally, HB may reduce the production of acetate (the precursor of milk fat) in the rumen (Farghaly et al., 2019), which can result in a reduction in milk fat synthesis.

Milk protein content decreased with dietary protein level in our study, a finding that is in agreement with Barros et al. (2017), who reported a linear decrease in milk true protein percentage from 3.58% to 3.33% as the CP content of TMR ration reduced from 16.2% to 11.8%. However, Chowdhury et al. (2023) reported negligible effect of dietary CP content (17.5% vs. 15.0%) on milk protein levels in cows fed diets based on lucerne or red clover, a variation that may be ascribed to the different nitrogen sources present in these diets. Additionally, the increase in milk protein yield in cows fed with HB at the high dietary CP level could also be attributed to the increase in milk production.

As the major bovine milk solid and osmotic regulator, the lactose content in milk has a low variability (Costa et al., 2019), although its concentration has been shown to be affected by dietary energy level. For example, Xue et al. (2011) reported that milk lactose concentration increased with increasing dietary concentrate level, while a low caloric density ration significantly reduced milk lactose (−0.15%) in the study of Beerda et al. (2007). Therefore, we speculate that the higher lactose content in cows fed the LP diet in the current study may relate to the greater energy intake.

Milk urea N concentration is an indicator of N use (Bobbo et al., 2020). A lower MUN content in cows fed the low-protein diets was observed in our study, which is in agreement with Chibisa and Mutsvangwa (2013), who reported that when dietary CP decreased from 17.2% to 15.0%, MUN concentration decreased from 14.8 mg/dL to 12.9 mg/dL. Rumen-derived NH_3-N, which is converted to urea-N in the liver, is the main source of MUN (Mutsvangwa et al., 2016). The high-protein diets were predicted to supply a greater content of RDP, which probably resulted in higher ruminal NH_3-N and led to the higher MUN concentration. Moreover, the predicted increase in the ruminal outflow of MP associated with high-protein diets could further contribute to the rise in MUN (Tebbe and Weiss, 2020). The reduction of MUN in the study of Chibisa and Mutsvangwa (2013) is higher than ours but still within our expected range, as the low dietary protein levels between studies were formulated by adjusting different protein feeds, and the type of protein may affect the metabolism of N in the rumen (Bach et al., 2005) and result in different magnitudes of MUN variation. As milk and milk component yield, in addition to DMI, were affected by a CP × HB interaction, then it is clear that the effect of HB is not consistent at different dietary protein levels, and that providing HB in a high-protein diet may be more beneficial to the performance of dairy cows.

Feed Efficiency and NUE

Lee et al. (2015) observed that cows that received MP-adequate diets tended to have a higher FE than those fed a MP-deficient diet. Gabler and Heinrichs (2003) also reported that the FE of Holstein heifers (ratio of kg of feed to kg of gain) improved linearly with dietary CP level. Feed efficiency also increased in cows fed the high-protein diets in our current study. However, the result should be interpreted with caution, as the improved FE in the above studies was mainly caused by an increased milk production or average daily gain, while it was mainly the lower feed intake in cows on high-protein diets compared with those in LP group that led to this result in our study. Reasons for these differences merits further investigation.

As dietary N intake decreases with the low-protein diet, the dependency of ruminal bacteria on urea-N increases. This urea-N is recycled from NH_3-N, which originates in the rumen and is subsequently processed in the liver (Mutsvangwa et al., 2016). Compared with the cows fed the high-protein diets, more recycled urea-N will be used for anabolic purposes to support microbial growth in response to the reduction in dietary N supply in cows fed the LP diets (Chibisa and Mutsvangwa, 2013). Microbial protein will be used as an amino acid source to the host animal, with deaminated amino acid-N converted to urea in the ornithine cycle and either recycled back to the rumen or excreted in urine (Mutsvangwa et al., 2016). Lee et al. (2011) also reported that urine N losses are more responsive to N intake than milk N secretion. Thus, NUE was improved by feeding the low-protein diets in the current study, which is in agreement with other findings (Mutsvangwa et al., 2016; Hynes et al., 2016).

Additionally, BUN content, a common indicator of NUE (Kohn et al., 2005), exhibits a positive correlation with CP intake and a negative correlation with NUE (Zhu et al., 2020; Lavery and Ferris, 2021; Chowdhury et al., 2023). In our study, the reduced BUN concentration in cows receiving the low-protein dietary treatments was associated with a higher NUE, reinforcing the relationship between dietary protein level and efficient nitrogen utilization.

Replacing 50% of CP of concentrate mixture with hydroponic barley in the ration of growing lambs improved nutrient utilization and N balance (Devendar et al., 2020). Hydroponic barley inclusion in the current study also resulted in a greater FE and NUE. The process of soaking, germination and sprouting often results in an increased content or availability of protein, carbohydrate, minerals and vitamins (Abouelezz et al., 2019), and the increased total VFA and propionate concentration from feeding HB reported by Farghaly et al. (2019) may have contributed to the increased FE in our study. Moreover, the increased energy metabolism in the rumen reflected in the higher total VFA concentration will have supported a greater microbial growth, thereby improving N utilization.

Blood Biochemical Parameters

Alanine aminotransferase plays an important role in the intermediate metabolism of glucose and amino acids (Sookoian and Pirola, 2012) and is found most abundantly in the cytosol of the hepatocyte, and little is released into blood, unless in the case of hepatocellular injury or death (Kim et al., 2008; Mohamed, 2014). An increased serum ALT concentration was detected in cows fed the low-protein diet in our current study, which is in accordance with the study of Zhu et al. (2020), who observed higher serum ALT content in goats fed a 12.0% CP diet than those fed a 14.8% CP diet. However, higher serum ALT content in the current study might not indicate liver damage because serum ALT concentrations were within the normal range (Andjelić et al., 2022).

Research by Niroula et al. (2019) and Nemzer et al. (2019) has shown that barley germination enhances its phenolic content and antioxidant activity. Lee et al. (2017) further discovered that barley sprout extract aids in protecting liver cells from oxidative stress by boosting glutathione synthesis in a mouse model. Additionally, Barbarestani et al. (2024) reported that including barley sprouts in the diet of aged broiler roosters (2% of their basal diet) not only reduced serum MDA concentrations but also upregulated the mRNA expression of GSH-Px and SOD. However, our study did not observe a similar influence on the activity of antioxidant enzymes in blood, which could be attributed to the rumen microbiota's involvement, and it has been suggested that polyphenolic compounds may be degraded or transformed into alternative complex structures in the rumen (Kim et al., 2021).

Nutrient Intake and ATTD

The intake of nutrients is directly related to the DMI of dairy cows and the nutritional level of the diet. Although the DMI has been discussed earlier, this section will focus on the apparent digestibility of nutrients.

As discussed, as barley grain germinates and develops into hydroponic barley, the nutrient availability is improved, resulting in an enhanced rumen fermentation (Farghaly et al., 2019), which may explain the increased DM and CP digestibility in cows fed HB at the high dietary CP level in our study. These results are also consistent with our findings on the production of dairy cattle, in that HB had no effect on DMI but increased the milk yield, FE, and NUE of cows fed the high-protein diet. It has been shown that ration RDP has a large influence on the production of rumen microbial crude protein, and is positively related to NDF and DM digestibility (NASEM, 2021). The RDP content of the LP diet was predicted to be lower than the HP diet, but cows in this group achieved a higher DM, CP, and ADF digestibility, which may be attributed to the higher DMI that supplied additional RDP to the rumen of LP cows. With the increased energy intake, this led to the milk yield, ECM production, 4% FCM output, and the yields of milk protein and lactose of LP being on par with or surpassing those observed in the HP group. Moreover, cows fed the LP diet exhibited a higher DMD and CPD, but lower FE. This pattern suggests that nutrients absorbed from digestion were not being efficiently allocated to milk production. We speculate that the low-protein diet may lead to a preferential redistribution of nutrients to body tissues rather than lactation, despite only a nominal increase in the BCS of these cows. This premise warrants further investigation.

CONCLUSIONS

Cows fed the high-protein diets exhibited a higher milk protein content and FE. Feeding the LP diet resulted in a higher DMI and milk lactose content, but a lower concentration of MUN and BUN. Additionally, NUE was increased by feeding the low-protein diets. Hence, feeding low-protein diets had no adverse effects on dairy cow performance. Regardless of the dietary protein level, the inclusion of HB improved FE and NUE. However, caution should be exercised when feeding HB with low-protein diets, as it led to reductions in DMI, milk yield, and milk fat yield. In contrast, when feeding with a high-protein diet, HB had no effect on DMI but increased milk production and elevated yields of milk protein and lactose. Therefore, incorporating HB into higher protein diets may support improvements in milk production performance. Moreover, further work on the effect of the inclusion of hydroponic barley on the rumen microbiota and metabolism may help to better explain the mechanism behind these results.

NOTES

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Nonstandard abbreviations used: AIA = acid-insoluble ash; ALB = albumin; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ATTD = apparent total-tract digestibility; CPD = ATTD of CP; CR = creatinine; DDGS = distillers dried grains with solubles; DMD = ATTD of DM; FE = feed efficiency; GSH-Px = glutathione peroxidase; HB = hydroponic barley sprouts; HP = high-protein diet, 16.8% CP; HP+HB = HP diet with hydroponic barley; LP = low-protein diet, 15.5% CP; LP+HB = LP diet with hydroponic barley; LSD = least significant difference; MDA = malondialdehyde; NUE = N use efficiency; TP = total protein; SOD = superoxide dismutase; T-AOC = total antioxidant capacity.

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