Reduction of crude protein in diets fed to lactating Holstein-Gyr cows

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Abstract – The objective of this work was to evaluate the effect of reducing crude protein (CP) contents in diets with a constant metabolizable protein content on the intake, performance, nitrogen balance, and nutrient digestibility of lactating Holstein-Gyr cows. Animals (n = 24, 103±23 days in milk) were allocated to four treatments (n = 6 per group) with different CP contents: 127, 132, 139, and 156 g kg^{-1} dry matter (DM). DM intake was not affected by treatments. CP intake and digestibility increased linearly with higher CP contents. Milk yield (23.7±3 kg per day) and the percentages of milk protein (3.3±0.2%) and fat (3.8±0.5%) were not affected by CP reduction. Milk and blood urea nitrogen increased linearly with the increase of CP in the diet, similarly to urinary nitrogen excretion. Nitrogen use efficiency was 29.8 and 22.4% when CP was 127 and 156 g kg^{-1} DM, respectively. Reducing CP in diets fed to mid-lactating Holstein-Gyr cows increases nitrogen use efficiency and maintains the productive performance of the cows.

Index terms: nitrogen, protected soybean meal, Soypass, urea.

Redução da proteína bruta em dietas de vacas Holandês-Gir em lactação

Resumo – O objetivo deste trabalho foi avaliar o efeito da redução da concentração de proteína bruta (PB) em dietas com mesmo teor de proteína metabolizável sobre o consumo, o desempenho, o balanço de nitrogênio e a digestibilidade de nutrientes em vacas Holandês-Gir, em lactação. Os animais (n = 24, 103±23 dias em lactação) foram alocados em quatro tratamentos (n = 6 por grupo) com diferentes teores de PB: 127, 132, 139 e 156 g kg^{-1} de matéria seca (MS). O consumo de MS não foi afetado pelos tratamentos. O consumo e a digestibilidade da PB aumentaram linearmente com a elevação dos teores de PB. A produção de leite (23,7±3 kg por dia) e os teores de proteína (3,3±0,2%) e de gordura no leite (3,8±0,5%) não foram afetados pela redução da PB. O nitrogênio uréico no leite e no plasma apresentou aumentos lineares com o aumento da PB nas dietas, assim como a excreção urinária de nitrogênio. A eficiência de utilização de nitrogênio foi de 29,8 e 22,4% quando a PB foi de 127 e 156 g kg^{-1} de MS, respectivamente. A redução da PB na dieta de vacas Holandês-Gir no terço médio da lactação aumenta a eficiência de utilização do nitrogênio e mantém o desempenho produtivo das vacas.

Termos para indexação: nitrogênio, soja protegida, Soypass, ureia.

Introduction

The intensification of milk production systems and the pursuit of maximum economic efficiency are a worldwide reality. With this focus, studies and investments have sought genetic improvement alongside herd nutrition to obtain highly productive animals. The crude protein (CP) requirements of these animals have been met by using 180 g kg^{-1} dry matter (DM) or more (Law et al., 2009). According to Law et al. (2009), an increase in CP of up to 173 g kg^{-1} DM in the diets of early-lactating cows had beneficial effects on milk production and DM intake. However, for animals between 151 and 305 days in milk, the most efficient nitrogen use was achieved with lower CP contents of 144 and 173 g kg^{-1} DM in the diets, with no detrimental effects on production (Law et al., 2009).

Environmental contamination with nitrogen has been an increasing concern of studies on animal production systems, especially on dairy farming.

Arriola Apelo et al. (2014) highlighted that, when dairy cows are fed diets that meet the metabolizable protein requirements recommended by Nutrient Requirements of Dairy Cattle (NRC, 2001), there is excess nitrogen and 75% of the ingested N is lost in urine and feces. This inefficiency increases the cost of protein supplementation and boosts the potential for environmental contamination with nitrogen. According to United States Environmental Protection Agency (USEPA, 2004), excess excreted nitrogen can cause the eutrophication and acidification of ecosystems. The goal of protein nutrition is to improve nitrogen use efficiency, which involves minimizing total protein intake, meeting the requirements for milk protein synthesis, as well as reducing feed costs and nitrogen excretion to the environment (Bahrami-Yekdangi et al., 2014).

Research on CP reduction in diets and on its impact on milk production has focused on indoor-housed Holstein cows with milk yield greater than 35 kg per day (Reed et al., 2017). The obtained results were consistent, indicating that CP levels between 16 and 16.5% are sufficient to maximize production (Fadul-Pacheco et al., 2017). Data are scarcer for crossbred cows raised in tropical environments; therefore, feed formulations are generally based on recommendations from international committees such as NRC (2001), which may result in diets with high CP and rumen-degradable protein, high excretion of nitrogen causing an environmental problem, and greater energy expenditure in the metabolism of excess nitrogen.

The objective of this work was to evaluate the effect of reducing crude protein contents in diets with a constant metabolizable protein content on the intake, performance, nitrogen balance, and nutrient digestibility of lactating Holstein-Gyr cows.

Materials and Methods

The study was approved by the ethics committee of Embrapa Gado de Leite (protocol number 14/2014), and was conducted at this corporation's experimental farm, located in Coronel Pacheco, in the state of Minas Gerais, Brazil.

Lactating non-pregnant Holstein-Gyr cows (n = 24; 15 primiparous and 9 multiparous) were used. The genetic composition of the cows varied between 3/4 and 7/8 Holstein and Gyr, respectively. At the beginning of the experiment, cows were 103±23 days in milk and had mean body weight (BW) of 484±46 kg and milk yield of 21±3 kg per day. All animals were housed in a free-stall barn with individual feed troughs controlled by the Calan Broadbent Feeding System (American Calan, Northwood, NH, USA).

Treatments consisted of four diets with different dietary CP concentrations: 127, 132, 139, or 156 g kg^{-1} DM, all with a constant metabolizable protein content, estimated at 1,888 g per day. The diets were formulated to be isoenergetic and were composed of corn silage, ground corn, soybean meal, Soypass (Cargill, Uberlândia, MG, Brazil), urea, and a mineral supplement (Table 1). Feed formulation was done using the Cornell Net Carbohydrate and Protein System, version 6.1 (Tylutki et al., 2008). The experiment followed a completely randomized design where the cows were distributed into four treatments (n = 6 per treatment), balanced according to days in milk, parity, genetic composition, milk production, and body weight.

During a 15-day pre-adaptation period, the cows received the control diet with 154 g CP per kilogram of DM. The trial was divided into two periods of 30 days each, totaling 60 experimental days. Each period consisted of 25 days of adaptation followed by 5 days of sampling. The animals were fed roughage and concentrate, which was mixed in the Data Ranger feed wagon (American Calan, NH, USA), twice a day in equal proportions, always after milking, with daily adjustment to maintain orts at 10%. Water was provided ad libitum.

Animals were milked twice a day at 06:00 and 15:00. Milk production was evaluated during three consecutive days in each of the nine experimental weeks using the DemaTron 75 automatic recorder (GEA Group AG, Düsseldorf, Germany). For compositional analysis, milk samples were collected weekly, at each milking, during the three consecutive days and stored in plastic vials containing 2-bromo-2-nitropropane-1,3-diol.

Milk production was corrected to 4% fat according to NRC (2001), using the formula $[(0.4 \times MP) + (15 \times \%F \times MP)]$, where MP stands for milk production and %F stands for milk fat percentage. Protein, fat, lactose, and blood urea nitrogen analyses were performed using the DairySpec FT component analyzer (Bentley Instruments Inc., Chaska, MN, USA), following the

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recommendations of International Dairy Federation (ISO, 2013).

In the last five days of each period, the differences between the diet provided and the orts were determined. For this, samples of concentrate, silage, and orts were collected and grouped into composite samples for each period. Dietary ingredients were sampled per treatment, in order to determine the actual composition of the diets during the experimental period (Table 1), whereas orts were sampled individually. Samples of corn silage and orts were stored at -20°C until further analysis.

During the last 12 days of each experimental period, 10 g titanium dioxide (TiO_2) were administered orally to the cows as a marker for the estimation of fecal production and apparent nutrient digestibility. Then, during the last 5 days of each period, after each milking, ten samples of 50 g feces were collected directly from the rectum, stored at -20°C, and pooled after thawing. Fecal production (grams per day) was estimated by dividing the amount of markers ingested (grams per day) by the fecal marker concentration (grams per day). The digestibility of dry matter, CP, neutral detergent fiber (NDF), and acid detergent fiber (ADF) was obtained using the formula:

[(amount consumed) - (amount of feces) / (amount consumed) x 100].

Nitrogen analysis was performed according to International AOAC (Windham, 1995). Nitrogen balance was obtained by subtracting the nitrogen excreted in feces, urine, and milk from the total nitrogen consumed. The excretion of nitrogen was calculated both for: feces, using daily fecal production, which was estimated by the titanium dioxide marker and nitrogen analysis; and urine, by subtracting the amount of nitrogen excreted in feces and milk from the nitrogen consumed.

Total urine excretion was estimated based on the daily urinary creatinine excretion, which was 1.06 mmol kg^{-1} metabolic BW (BW0.75) (Chizzotti et al., 2008). The total excretion of purine derivatives (PD) was calculated as the sum of allantoin and uric acid excreted in urine and milk. Absorbed purines (AP) were determined from the excretion of PD (mmol per day) and BW (kg), through the equation: AP (mmol per day) = (PD + 0.385 × BW0.75)/0.85, where 0.85 represents the recovery of AP as PD and 0.385 ×

Table 1. Ingredients and chemical composition of the evaluated diets.

Item	Crude protein concentration (g kg^{-1} DM)			
	124	130	136	154
Ingredients (g kg^{-1})				
Corn silage	530	533	540	536
Ground corn	323	324	316	296
Soybean meal	53	70	83	133
Urea	0	29	57	87
Soypass(1)	68	44	29	0
Sodium bicarbonate	7	7	7	7
Mineral-vitamin supplement(2)	19	19	19	19
Chemical composition				
Dry matter (DM, g kg^{-1} organic matter)	585	583	580	582
Crude protein (g kg^{-1} DM)	127	132	139	156
Gross energy (kcal kg^{-1})	3.96	3.95	3.94	3.94
Non-fiber carbohydrates (g kg^{-1} DM)	438	435	427	413
Neutral detergent fiber (g kg^{-1} DM)	358	355	356	353
Acid detergent fiber (g kg^{-1} DM)	162	161	161	161
Ether extract (g kg^{-1} DM)	33	33	33	33
Rumen-degradable protein (g kg^{-1} DM)(3)	56	67	77	97
Rumen-undegradable protein (g kg^{-1} DM)(3)	68	63	59	57
Metabolizable protein (gram per day)(3)	1.88	1.88	1.88	1.88
Net energy for lactation (Mcal kg^{-1} DM)(3)	1.61	1.62	1.62	1.61

(1)Lignosulfonate-treated soybean meal (Cargill, Uberlândia, MG, Brazil). (2)Composition (kilogram of dry matter): 190 g Ca, 60 g P, 20 g S, 20 g Mg, 35 g K, 70 g Na, 15 mg Co, 700 mg Cu, 10 mg Cr, 700 mg Fe, 40 mg I, 1,600 mg Mn, 19 mg Se, 2,500 mg Zn, 200,000 IU vitamin A, 50,000 IU vitamin D3, 1,500 IU vitamin E, and 600 mg F. (3)Estimated using the Cornell Net Carbohydrate and Protein System, version 6.1 (Fox et al., 2004).

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BW0.75 represents the endogenous contribution to purine excretion (Verbic et al., 1990). Microbial protein synthesis was calculated based on AP, as: microbial nitrogen (gram of nitrogen per day) = 70X / 0.116 × 0.83 × 1,000, where X stands for AP (Chen & Gomes, 1992). Nitrogen efficiency (NE) was measured by the relationship between the nitrogen present in milk and the nitrogen consumed (NE = nitrogen in milk/ nitrogen consumed).

Blood samples of 5 mL were collected by coccygeal vessel puncture, on the last day of each experimental period, 4 hours after morning feeding, using BD Vacutainer tubes containing EDTA and sodium fluoride (BD no Brasil, São Paulo, SP, Brazil). The samples were immediately centrifuged at 800 *g* for 15 min in the equipment Excelsa II model 206-BL (Fanem, São Paulo, SP, Brazil), and plasma was stored at -20°C. Plasma glucose and blood urea nitrogen analyses were performed using the Labmax Premium 240 automated monochrome reading system and a commercial kit (Labtest, Lagoa Santa, MG, Brazil).

Samples of silage, orts, and feces were dried in a forced-air ventilation oven, at 55°C, for 72 hours. Subsequently, the samples were ground to 1-mm particle size in a mill for determination of DM, ash, CP, gross energy (GE), NDF, ADF, and ether extract. DM, CP, and ash were analyzed according to International AOAC (Windham, 1995), and NDF and ADF, using the method described by Van Soest et al. (1991). GE was determined in the IKA C500 adiabatic calorimeter (IKA Werke GmbH & CO. KG, Staufen, Germany), whereas non-fiber carbohydrates (NFC) were obtained by the difference between total carbohydrates (tCHO) and NDF (tCHO = 100 - %EE - %CP - %ASH). Metabolizable protein, rumen-degradable protein, rumen-undegradable protein, and net energy for lactation were estimated according to the Cornell Net Carbohydrate and Protein System (Fox et al., 2004).

Data were analyzed using the SAS software, version 9.0 (SAS Institute Inc., Cary, NC, USA). Feed intake and milk production and composition were evaluated through mixed models (Proc Mixed) for repeated measures, with treatment, period, and treatment x period interaction as fixed effects, and cow within treatment as a random effect. Blood parameters, nitrogen use efficiency, and nutrient digestibility were also analyzed using mixed models (Proc Mixed), but in subdivided plots, where CP concentration was considered as a plot and periods (days in milk) as subplots, including the effect of treatment, period, and treatment x period interaction as fixed effects, and cow within treatments as a random effect. The differences between treatments and BW changes during the experiment were assessed by the orthogonal polynomial contrasts used to estimate the linear, quadratic, and cubic effects of increasing dietary CP for lactating cows. Individual milk yields and BW before the start of the experiment were used as covariates. The differences between means were considered significant at 5% probability.

Results and Discussion

The intakes of DM, NDF, ADF, EE, and GE were similar among treatments (Table 2), possibly because the diets were isoenergetic and balanced to meet the metabolizable protein requirements of the Holstein-Gyr cows. CP intake increased linearly, which may be justified by its increased levels in the treatments.

The digestibility of DM, NDF, and ADF did not differ among treatments (Table 2). The digestibility of CP increased linearly according to the increase in its contents, probably due to a dilution effect, given that fecal nitrogen remained constant among treatments. Edouard et al. (2016) also found that different CP contents resulted in similar excretions of fecal nitrogen, but differed regarding the excretion of urea nitrogen. In addition, Bahrami-Yekdangi et al. (2014), when evaluating the addition of 156 to 180 g CP per kilogram of DM in the diets of dairy cattle, reported greater apparent protein digestibility for treatments with higher CP concentrations. These results show that reducing nitrogen intake is a strategy for the reduction of nitrogen excretion.

Milk production and composition were not affected by dietary CP concentrations (Table 3). The percentage of lactose in milk had a quadratic effect on CP increase, and, although lactose production was similar among treatments, an optimum point was observed at 147 g CP per kilogram of DM. The predicted quantity of metabolizable protein was met and constant at all protein levels in the experimental diets (1,888 g per day), meeting the protein requirements of the cows. Diets with lower CP contents showed the highest rumen-undegradable protein values (Table 1), which met the metabolizable protein requirements of the animals, as observed in the other treatments. In the present study, rumen-degradable protein concentrations ranged from 56 to 97 g kg^{-1} DM and did not limit or alter milk production or composition, respectively. In other studies, however, milk production (Cyriac et al., 2008; Lee et al., 2011), milk protein concentration, or protein production were decreased when dietary CP was reduced (Imaizumi et al., 2010; Lee et al., 2012).

Mean milk production was 23.7±3 kg per day, showing that, in this range, lower dietary CP concentrations did not limit the productive performance of the animals, which is in alignment with Gressley & Armentano (2007) and Agle et al. (2010). Despite the variation in dietary CP concentrations, the constant metabolizable protein was able to maintain regular milk, lactose, fat, and protein production, as well as milk fat and protein contents. According to Law et al. (2009), mid- and late-lactating Holstein cows can be fed diets with reduced CP content without losses in productive performance, leading to a reduction in feed cost as protein is one of the most expensive nutrients for lactating cows. For the Holstein-Gyr cows evaluated in the present study, the low levels of CP did not entail losses in performance;

Table 2. Intake and digestibility of nutrients consumed by lactating Holstein-Gyr dairy cows fed different crude protein (CP) concentrations.

Item	CP concentration (g kg^{-1} DM)				SEM(1)	P-value(2)		
	127	132	139	156		T	W	TxW
Intake								
DM (kg per day)	20.0	20.8	21.4	21.6	0.30	0.25	0.43	0.92
CP (kg per day)	2.6	2.8	3.0	3.4	0.06	0.01(3)	0.23	0.78
NDF (kg per day)	7.0	7.3	7.5	7.4	0.11	0.38	0.01	0.95
ADF (kg per day)	3.1	3.1	3.2	3.1	0.06	0.83	0.01	0.99
EE (kg per day)	0.7	0.7	0.7	0.7	0.01	0.60	0.01	0.95
GE (Mcal per day)	78,952	81,978	84,213	84,440	1,209	0.34	0.11	0.93
Digestibility (%)								
DM	61.1	60.2	61.9	60.7	0.90	0.93	0.15	0.35
CP(3)	58.2	60.7	62.9	67.2	1.00	0.04	0.16	0.23
NDF	43.8	41.9	41.8	43.8	1.27	0.91	0.36	0.91
ADF	34.2	31.5	31.5	35.0	1.50	0.81	0.98	0.86

(1)Standard error of the mean. (2)T, treatment effect; W, week effect; and TxW, treatment x week interaction. (3)Linear effect ($p \leq 0.01$; $y = 0.2619x - 0.6119$; $R^2=0.98$). DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extracts; and GE, gross energy.

Table 3. Milk production, components, and composition, as well as glucose and blood urea nitrogen, of lactating Holstein-Gyr dairy cows fed different crude protein (CP) concentrations.

Item	CP concentration (g kg^{-1} DM)				SEM(1)	P-value(2)		
	127	132	139	156		T	W	TxW
Milk yield (kg per day)	24.5	22.4	24.0	23.8	0.22	0.54	0.07	0.12
4% fat corrected milk (kg per day)	23.9	21.5	23.7	22.8	0.22	0.34	0.18	0.17
Milk fat (%)	3.9	3.7	3.9	3.8	0.03	0.89	0.01	0.88
Milk fat (g)	955	834	955	862	10.32	0.23	0.03	0.31
Milk true protein (%)	3.3	3.2	3.4	3.3	0.02	0.82	<0.01	0.07
Milk true protein (g per day)	810	725	815	789	7.71	0.23	0.01	0.35
Milk lactose (%)	4.4	4.6	4.7	4.7	0.01	0.01(3)	0.03	0.23
Milk lactose (g per day)	1,085	1,030	1,132	1,121	10.19	0.46	0.02	0.18
Milk urea nitrogen (mg dL^{-1})	8.1	12.2	14.4	18.5	0.32	0.01(4)	0.01	0.39
Glucose (mg dL^{-1})	59.0	57.0	57.0	60.0	0.76	0.33	0.61	0.59
Blood urea nitrogen (mg dL^{-1})	10.2	13.9	15.6	19.6	0.75	<0.01(5)	<0.01	0.06

(1)Standard error of the mean. (2)T, treatment effect; W, week effect; and TxW, treatment x week interaction. (3)Quadratic effect ($p \leq 0.01$; $y = -0.076x^2 + 2.2032x - 11.186$, $R^2=0.99$). (4)Linear effect ($p \leq 0.01$; $y = 3.2262x - 30.576$, $R^2=0.93$). (5)Linear effect ($p \leq 0.01$; $y = 3.2262x - 30.576$, $R^2=0.93$).

therefore, the reduction in nitrogen content is a strategy to reduce nitrogen excretion and feed costs in tropical dairy production systems.

Cows fed higher CP concentrations excreted greater amounts of urea nitrogen in milk (linear effect, $p<0.01$), indicating inefficiency in the use of dietary nitrogen for productive purposes with increased CP. Similar results were reported by Danes et al. (2013) and Arriola Apelo et al. (2014). Feeding excess nitrogen requires the use of additional energy to metabolize excess protein as observed by Reed et al. (2017), who found increases in heat production and decreases in energy retained in tissue and in milk gross energy after Holstein cows were fed diets with excess nitrogen.

Plasma glucose concentration (mg dL^{-1}) was similar among cows receiving diets with different CP concentrations, and the mean glucose content was 58±5 mg dL^{-1}. Likewise, Bahrami-Yekdangi et al. (2014) also did not report effects of dietary CP on plasma glucose concentrations, which ranged from 58 to 60 mg dL^{-1}, when evaluating the effects of reducing CP from 180 to 156 g kg^{-1} DM in the diet of early-lactating Holstein cows.

Blood urea nitrogen differed among treatments (Table 3). A linear increase ($p<0.01$) was verified with the increase in CP concentrations, as observed for milk urea nitrogen. According to Nousiainen et al. (2004), blood urea nitrogen is the main end product of nitrogen metabolism in the body and it rapidly balances with body fluids. The high levels of blood urea as a response to the increase in CP could be associated with efficiency losses due to the additional energy necessary to metabolize excess protein as verified by Reed et al. (2017).

The increase in dietary CP concentration was associated with a linear increase in the amount of ingested nitrogen (Table 4). Nitrogen in milk was equal among experimental groups, showing that the nitrogen requirement of all animals for milk production and protein was met, which can be explained by the constant metabolizable protein used for all treatments (Table 1). The decrease in rumen-degradable protein could have been a limiting factor for the synthesis of microbial protein in the rumen and could have influenced the amount of microbial nitrogen that would reach the intestine. However, in the present study, this is unlikely, given that the synthesis of microbial protein in the rumen for all treatments met the nutritional requirements of mid-lactating Holstein-Gyr cows producing 23.7 kg per day (NRC, 2001).

The mean fecal nitrogen excretion was 179±33 g per day and did not differ among treatments, corroborating the results of Edouard et al. (2016) and Bahrami-Yekdangi et al. (2014), who found similar excretions of fecal nitrogen with varying CP contents. The nitrogen excreted in feces originates from indigestible microbial protein produced in the rumen, endogenous proteins, and indigestible dietary protein. The variation in rumen-undegradable protein (Table 1) could have affected mainly the amount of indigestible dietary protein, but this fraction is less representative than the total fecal nitrogen excreted (Davidson et al., 2003).

The amount of nitrogen excreted in urine increased linearly according to the increase in CP. Since nitrogen is rapidly converted to ammonia, which volatilizes and

Table 4. Nitrogen balance and nitrogen use efficiency in lactating Holstein-Gyr dairy cows fed different crude protein (CP) concentrations.

Item	CP concentration (g kg^{-1} DM)				SEM(1)	P-value(2)		
	127	132	139	156		T	W	TxW
N intake (g per day)	414	452	490	553	9.21	0.01	0.23	0.78
Milk N (g per day)	125	119	135	123	2.84	0.12	0.30	0.45
Fecal N (g per day)	178	179	182	180	4.93	0.99	0.54	0.39
Fecal N:N intake(2)	42	39	37.0	33	0.01	0.04	0.16	0.23
Urinary N (g per day)(3)	116	155	172	247	8.46	0.01(2)	0.21	0.21
Urinary N:N intake(2)	29	33	35	45	0.01	0.01	0.18	0.20
Microbial N	356	394	468	346	23.4	0.13	0.04	0.29
N efficiency(4)	29.8	26.5	27.5	22.4	0.006	0.01(2)	0.65	0.60

(1)Standard error of the mean. (2)T, treatment effect; W, week effect; and TxW, treatment x week interaction. (2)Linear effect ($p\leq0.05$; $y = 42.143x - 400.64$, $R^2=0.99$). (3)Predicted urinary N (gram per day) = N intake (gram per day) - milk N (gram per day) - fecal N (gram per day). (4)Nitrogen use efficiency (%) = 100 × milk N (gram per day)/N intake (gram per day).

reaches the atmosphere, this is an important factor for environmental pollution (Dijkstra et al., 2011). Increased nitrogen excretion to the environment is a growing concern of dairy farmers, because animal production systems have been blamed of causing an environmental impact. Moreover, the inefficiency in protein metabolism may represent unnecessary costs to farmers and can impair animal performance due to negative impacts on reproduction and energy expenditure to eliminate excess nitrogen from the body (Reed et al., 2017).

There was no effect of different concentrations of dietary CP on microbial nitrogen production (Table 4), as observed by Agle et al. (2010). Reynolds & Kristensen (2008) suggested that diets with lower CP contents have both greater urea recycling and supply of additional rumen-degradable protein for ruminal microorganisms to carry out microbial protein synthesis.

Net energy can be used as a tool for environmental and feeding management in dairy farms, and may also enhance farm profits when improved (Fadul-Pacheco et al., 2017). The dietary nitrogen input is the primary factor determining net energy (Huhtanen & Hristov, 2009). In the present study, there was a linear reduction in net energy of 30 and 22.5% as CP increased from 127 to 156 g kg^{-1} DM, respectively. Lower CP contents and rumen-degradable protein show environmental and metabolic advantages due to lower nitrogen excretion and lower energy expenditure for the metabolism of extra nitrogen (Reed et al., 2017). Supporting these findings, Fadul-Pacheco et al. (2017), grouping 100 Québec dairy herds based on their net energy, found differences in CP concentrations of 16.0 vs 14.2 g kg^{-1} DM between clusters of high (36%) and low (22%) net energy.

Conclusions

1. The increase in crude protein concentrations does not influence milk, fat and protein production of mid-lactating Holstein-Gyr cows, but it increases the excretion of urinary nitrogen and provides lower dietary nitrogen for milk protein conversion efficiency.

2. The use of lower amounts of crude protein in the diets of mid-lactating Holstein-Gyr cows is an alternative to minimize the environmental impacts associated with the excretion of nitrogenous compounds.

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