# Levels and degradability of crude protein in digestive metabolism and performance of dairy cows

## Níveis e degradabilidade da proteína bruta no metabolismo digestivo e desempenho de vacas leiteiras

Bruna Gomes Alves[1]; Cristian Marlon de Magalhães Rodrigues Martins[1]; Dannylo de Oliveira Sousa[2]; Marcos André Arcari[1]; Francisco Palma Rennó[1]; Marcos Veiga Santos[1]

[1] Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Nutrição e Produção Animal, Pirassununga – SP, Brazil
[2] Swedish University of Agricultural Sciences, Department of Animal Environment and Health, Vastra Gotaland, Sweden

**ABSTRACT**

Two experiments were conducted to evaluate the effect of the level and degradability of crude protein (CP) on the digestive metabolism and productive performance of dairy cows. In both experiments, 15 Holstein cows with $585 \pm 40$ kg of body weight were distributed in a Latin square design with five contemporary squares, three periods of 21 days and three treatments. In experiment 1, treatments consisted of three CP levels (130, 160 or 180 g CP/kg DM), while in experiment 2, the treatments consisted of three levels of rumen degradable protein (RDP; 80, 100 or 120 g RDP/kg DM) in diets with average of 163 g CP/kg DM. Variables evaluated in both experiments were dry matter intake (DMI), total apparent digestibility, milk yield (MY) and composition, ruminal fermentation and N balance. In experiment 1, the increase of CP from 130 to 180 linearly increased the organic matter, CP, neutral detergent fiber (NDF) and acid detergent fiber (ADF) intake (kg) and the apparent total digestibility coefficient of DM and CP. In addition, a linear increase of MY, fat corrected milk (FCM) and daily production of fat, protein, lactose, casein and total solids was observed. A linear increase in ruminal ammoniacal nitrogen ($NH_3$-N) concentration and nitrogen excretion in milk, feces and urine was also observed. However, there was no observed effect on SCFA concentration. In experiment 2, the increase of the RDP from 80 to 120 increased the DMI, MY, FCM, milk protein content and digestibility coefficient of the NDF, ADF and ethereal extract. Additionally, there was an increase in $NH_3$-N concentration and milk nitrogen excretion. The studies indicated that the increase of CP content up to 100 g RDP/kg DM increased the DMI and the productive performance of the cows, but also increased urine N. Thus, it is desirable that the increase of the CP through the increase of the RDP is carried out up to 100 g of RDP/kg DM, since there is elimination of nitrogen, decrease of milk yield and decrease of propionic acid in values above that level.

**Keywords:** Digestibility. N balance. Protein. Rumen-degradable protein. Ruminal fermentation.

**RESUMO**

Dois experimentos foram realizados com o objetivo de avaliar o efeito do nível e da degradabilidade da proteína bruta (PB) no metabolismo digestivo e no desempenho produtivo de vacas leiteiras. Nos dois experimentos, 15 vacas da raça Holandesa com $585 \pm 40$ kg de peso corporal foram distribuídas em delineamento do tipo quadrado latino com cinco quadrados contemporâneos, sendo três períodos de 21 dias e três tratamentos. No experimento 1, os tratamentos consistiram de três níveis de PB (130, 160 ou 180 g CP/kg de MS), enquanto que no experimento 2, os tratamentos consistiram de três níveis de proteína degradável no rúmen (PDR; 80, 100 ou 120 g de PDR/kg MS) em dietas com média de 163 g CP/kg MS. As variáveis avaliadas nos dois experimentos foram: consumo de matéria seca (CMS), digestibilidade aparente total, produção e composição do leite (PL), fermentação ruminal e balanço de N. No experimento 1, o aumento da PB de 130 para 180 aumentou linearmente o consumo de matéria orgânica, PB, fibra detergente neutro (FDN), fibra em detergente ácido (ADF) (kg) e o aparente coeficiente de digestibilidade total de MS e PB. Além disso, foi observado um aumento linear da PL, leite corrigido para gordura (LCG) e produção diária de gordura, proteína, lactose, caseína e sólidos totais. Também foi observado aumento linear na concentração de nitrogênio amoniacal ruminal (N-$NH_3$) e excreção de nitrogênio no leite, fezes e urina. No entanto, não foi observado efeito na concentração de ácidos graxos de cadeia curta. No experimento 2, o aumento do PDR de 80 para 120 aumentou o CMS, PL, LCG, teor de proteína do

leite e o coeficiente de digestibilidade do FDN, FDA e extrato etéreo. Além disso, houve aumento na concentração de N-NH$_3$ e excreção de nitrogênio no leite. Os estudos indicaram que o aumento do teor de PB em até 100 g RDP/kg de MS aumentou o CMS e o desempenho produtivo das vacas, mas também aumentou o N urinário. Assim, é desejável que o aumento da PB através do aumento da PDR seja realizado até 100 g de PDR/kg de MS, uma vez que há eliminação de nitrogênio, diminuição da produção de leite e diminuição do ácido propiônico em valores acima desse nível.

**Palavras-chave:** Digestibilidade. Balanço de N. Proteína. Proteína degradável no rúmen. Fermentação ruminal.

**Correspondence to:**
Marcos Veiga Santos
Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Nutrição e Produção Animal
Av. Duque de Caxias Norte, 225
CEP: 13635-900, Pirassununga – SP, Brazil
e-mail: mveiga@usp.br

## Introduction

When dairy cows are fed unbalanced diets, the dietary protein is not effectively used, generating an excess nitrogen (N) that is excreted in feces and urine and negatively affects the environment and economics of dairy farms (Mutsvangwa et al., 2016). The N excreted by ruminants can be converted into multiple forms, such as ammonia, N$_2$O (a potential greenhouse gas) and nitrate, a water pollutant (Kebreab et al., 2001). Previous studies indicated that increasing CP supply from 16.5 to 18.5% increased milk yield and milk protein content (Bahrami-Yekdangi et al., 2014; Broderick, 2003) but with loss in efficiency of N use and consequently with greater excretion in the environment (Colmenero & Broderick, 2006b; Rius et al., 2012). On the other hand, the use of diets with lower levels of CP or rumen degradable protein (RDP) increases the efficiency of N and meets the requirements of microbial and metabolizable protein and has no negative effects on milk yield (MY).

Increased N efficiency and microbial protein synthesis can be achieved by optimizing the RDP: RUP ratio (Kalscheur et al., 2006; Wang et al., 2007), by supplying limiting essential amino acids in diets (Broderick et al., 2009; Lee et al., 2012), and the good level of rumen carbohydrate fermentation, mainly starch. The requirements of amino acids are met by rumen-undegradable protein (RUP) while the increasing rumen starch digestibility may increase microbial protein synthesis due to greater supply of substrates (fermentable carbohydrates) for microorganism growth (Vaz-Pires et al., 2008). The optimization of RDP:RUP ratio determines the quantity and quality of proteins and amino acids available to the cow and, to meet the requirements without excessive N, rations must also be balanced for RUP and RDP.

Corn grain, the main energy source used in ruminant diets, is resistant to bacterial fermentation by the presence of a protein matrix that surrounds the starch granule. Thus, several strategies can be adopted to increase protein and starch ruminal digestibility, such as milling, lamination, flocculation and ensilage (high moisture and rehydrated corn). In addition, rehydrated corn silage increases this digestibility through microbial enzymatic degradation with low cost when compared to the lamination and flocculation processes (Arcari et al., 2016; Vaz-Pires et al., 2008). Considering amino acid requirement, it is possible that RUP sources can promote better productive responses when the forage is sugarcane, since shows an imbalance of nutrients and is usually only supplemented with non-protein nitrogen (NPN).

So, our hypothesis was that optimum levels of CP and RDP:RUP optimize the productive performance and N use efficiency. Thus, the aim of the present study was to evaluate the effect of three dietary CP and RDP levels on digestive metabolism, productive performance and N use efficiency of cows fed diets based on rehydrated corn and sugarcane silage.

## Materials and Methods

This study was approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science (CEUA/FMVZ/USP - protocol number 2671/2012).

### Diets and feeding management

Two experiments were conducted sequentially to assess changes in crude protein content and subsequently in PDR levels. For both experiments, 15 Holstein cows averaging 150 (± 60 SD) DIM (days in milk), body weight (BW) of 585 (± 40 SD) kg and MY of 30 (± 4.13 SD) kg/day were used. The cows were milked twice a day (07:00

and 16:00) and the intake control was performed daily. The facilities were individual pens of approximately 16 m². Five 3 × 3 contemporary Latin square was used as experimental design with 21-day periods, and each square was blocked according to MY, BW and parity. The first 14 days were used for the cows' adaptation to the diets and the last 7 for the evaluation of DMI, MY, milk composition, total apparent digestibility of nutrients, diet composition and ruminal metabolism. The experiments were conducted sequentially, with experiment 2 following experiment 1.

In experiments 1 and 2, the diets were formulated to be isoenergetic (1.57 NE$_l$/kg DM and 1.55 NE$_l$/kg DM, respectively). Three CP levels (130, 160, and 180 g of CP/kg of DM) were done in trial 1, while in trial 2, the diets also were formulated to be isonitrogenous (163 g CP/kg DM), but with three RDP contents (80, 100, and 120 g of RDP/kg of DM). Both experiments used sugarcane silage as roughage, soybean meal, urea, rehydrated corn silage, corn gluten meal and mineral and vitamin supplement(Table 1). The amount of feed offered was calculated to allow daily orts of 5 to 10%.

Sugarcane (*Saccharum officinarum* L.) variety IAC86-2480 (IAC/APTA, São Paulo, Brazil) was harvested by a forage harvester (Colhiflex; Menta Mit, Cajuru, SP, Brazil) adjusted for an average particle size of 10.8 mm (Lammers et al., 1996). The silage microbial inoculant (*Lactobacillus buchneri*, LalSil® Cana - Lallemand, Brazil) was used at a concentration of 2g/ton, by spraying a diluted solution (100g/50L of water) to achieve a standard rate of 4×10⁵ CFU/g of fresh forage that was kept ensiled for 60 d before opening. The corn grains (variety Dow 2B710 HX°; Dow AgroScience, Mogi Mirim, SP, Brazil) were processed, rehydrated and inoculated according Arcari et al. (2016).

### Feed and feces analysis and total digestibility

The samples of the feed ingredients, total diets, feces and orts were collected as proposed by Arcari et al. (2016) to determine the nutrients intake of dry matter, organic matter (OM), CP, NDF, ADF and ethereal extract (EE). They were stored at -20 °C until laboratory analysis.

Table 1 – Ingredients and chemical composition of diets with different levels of crude protein and protein degradability in the dairy cows rumen

| Item | CP (g/kg DM) | | | RDP (g/kg DM) | | |
|---|---|---|---|---|---|---|
| | 130 | 160 | 180 | 80 | 100 | 120 |
| **g/kg DM** | | | | | | |
| SCS[a] | 399.0 | 400.8 | 400.6 | 400.5 | 402.3 | 399.8 |
| RCS[b] | 444.2 | 388.7 | 320.5 | 358.5 | 387.2 | 429.4 |
| Soybean meal | 89.9 | 120.8 | 180.5 | 120.2 | 120.8 | 120.0 |
| Corn gluten meal | 21.2 | 40.4 | 50.5 | 85.0 | 40.4 | - |
| Urea | 6.7 | 8.8 | 8.0 | - | 8.8 | 17.9 |
| Mineral mix[c] | 23.4 | 24.9 | 24.3 | 21 | 24.9 | 22.3 |
| Ammonium sulfate | 1.0 | 1.0 | 1.0 | 1.6 | 1.0 | 1.7 |
| Sodium bicarbonate | 8.8 | 8.8 | 8.8 | 8.0 | 8.8 | 8.5 |
| Magnesium oxide | 1.6 | 1.6 | 1.6 | 1.4 | 1.6 | 1.5 |
| Dicalcium phosphate | 2.1 | 2.1 | 2.1 | 2.4 | 2.1 | 2.6 |
| Sodium Chloride | 2.1 | 2.1 | 2.1 | 1.4 | 2.1 | 1.5 |
| **Chemical composition g/kg DM** | | | | | | |
| Dry matter | 434.4 | 439.6 | 435.6 | 470.8 | 470.4 | 470.3 |
| NE$_L$ (Mcal/kg)[d] | 1.56 | 1.58 | 1.57 | 1.58 | 1.55 | 1.53 |
| Mineral matter | 66.2 | 62.3 | 67.6 | 62.1 | 60.5 | 63.2 |
| Crude protein | 132.0 | 161.0 | 187.0 | 163.1 | 162.8 | 163.2 |
| Starch | 306.4 | 268.2 | 221.1 | 247.3 | 267.1 | 272.3 |
| Ethereal extract | 29.0 | 27.0 | 25.0 | 27.0 | 27.0 | 28.0 |
| NDF[e] | 320.0 | 327.0 | 329.0 | 328.0 | 327.0 | 325.0 |
| ADF[f] | 199.0 | 204.0 | 207.0 | 206.0 | 204.0 | 203.0 |
| Lignin | 30.0 | 29.0 | 28.0 | 31.0 | 30.0 | 32.0 |
| RDP[g] | 82.0 | 100 | 116 | 80.0 | 100.0 | 120.0 |
| RUP[g] | 50.0 | 61.0 | 71.0 | 83.1 | 62.8 | 43.2 |
| RDP: RUP ratio | 1.64 | 1.64 | 1.64 | 0.97 | 1.59 | 2.77 |

DM: dry matter; CP: crude protein; [a]SCS: Sugarcane silage; [b]RCS: Rehydrated corn silage = grain corn with particle size of 1,681µm ensiled with 65% DM for 100 days prior delivery; [c]per Kg: calcium – 190 g, phosphor – 73 g, sulfur – 30 g, magnesium – 44 g, copper – 340 mg, zinc – 1350 mg, manganese – 940 mg, cobalt – 3 mg, iodine – 16 mg, selenium – 10 mg, iron – 1064 mg, vitamin A - 100.000 IU, vitamin D - 40.000 IU, vitamin E - 600 IU; NE$_L$= net energy from National Research Council (2001); [e]NDF = neutral detergent fiber; [f]ADF = acid detergent fiber; [g]RDP = rumen-degradable protein; RUP = rumen-undegradable protein from National Research Council (2001).

After thawing at room temperature, samples were pre-dried in a forced air oven (60 °C for 72 h) and milled in 1 mm porosity sieves (Marconi, MA 048 model). The analysis of dry matter, lignin, ethereal extract (EE) and ash were done according Association of Official Analytical Chemists (1990), described in more detail in Arcari et al. (2016). Concentrations of NDF and ADF were determined according to Van Soest et al. (1991) and total N content was determined based on DM basis for silage and concentrate and based on OM for feces and urine (Elementar Rapid N, method 990.03, Association of Official Analytical Chemists, 2000). The NDF, starch content and rehydrated corn silage analysis, including pH and particle size were determined using the same methodology described by Arcari et al. (2016). Geometric mean particle size (GMPS) evaluation was performed according Baker & Hermann (2002) (Table 2).

The estimate of total fecal excretion and the total apparent digestibility of individual feeds, orts and feces were done as described by Arcari et al. (2016), using the indigestible acid detergent fiber (iADF) as an internal marker.

### Milk sampling and analyses

The milk production was daily measured in the last 7 days of each experimental period by an electronic system (DeLaval, Campinas, Brazil). The fat milk production corrected to 3.5% (FCM) was calculated according to Sklan et al. (1994). Milk samples were collected from d 16 to 19 of each period, refrigerated and preserved with 2-bromo-2-nitropropane-1,3-diol (0.05% w/v) and analyzed by infrared absorption (Bentley Instruments, 1995) for determination of fat, protein, lactose, total solids and casein. Analyses of urea nitrogen in milk were performed using an automated system based on the enzymatic spectrophotometric method (ChemSpeck 150®, Bentley Instruments, Chaska, Minnesota, USA).

Table 2 – Chemical composition of dry corn, rehydrated corn silage, sugarcane silage and soybean meal of diets for dairy cows

| Nutrient | GDC | RCS | SCS | SM |
|---|---|---|---|---|
| | *Chemical composition* | | | |
| DM g/kg | 870.0 | 670.0 | 310.7 | 875.3 |
| CP g/kg MS | 81.0 | 82.0 | 40.0 | 500.0 |
| SP, % CP | 6.0 | 33.1 | - | - |
| Starch g/kg DM | 693.0 | 695.0 | 10.0 | 40.0 |
| NDF | 16.9 | 16.8 | 593.1 | 17.7 |
| ADF | 3.3 | 3.2 | 460.3 | 10.5 |
| pH | 6.1 | 3.9 | 4.0 | - |
| GMPS (μm) | - | 1.0 | - | - |

GDC: ground dry corn; RCS: rehydrated corn silage; SCS: sugarcane silage; SM: soybean meal; -: not analyzed; DM: dry matter; CP: crude protein; SP: soluble protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; GMPS: geometric mean particle size.

### Rumen fluid and urine analyses

In the last days of collection of each experimental period, collections of ruminal liquid were carried out through esophageal probe and urine, collected after vulvar stimulation, as described by Arcari et al. (2016). The ruminal samples were processed for determination of ammoniacal nitrogen (N-NH$_3$), N balance (ingested - NI, feces - NF, urine - NU; total - NT) and short-chain fatty acids (SCFA). Urine analyses were performed to pH, creatinine concentrations, estimative of total daily urine volume and the urinary creatinine excretion. Both analyses were done as described by Martins et al. (2016).

### Statistical analysis

The data were analyzed using the MIXED procedure of SAS version 9.3 (SAS Institute Inc., 1995), after verification of the residual normality and variance homogeneity. The main effects of the treatments were evaluated according to the following model: $Y_{ijkl} = \mu + T_i + Q_j + A(Q)_k + P_l + e_{ijkl}$, where $Y_{ijkl}$ = is the observed value; $\mu$ = overall mean; $T_i$ = fixed treatment effect i; $Q_j$ = random effect of Latin square j, j = 1 to 3; $A(Q)_k$ = random effect of animal k within each Latin square, k = 1 to 15; $P_l$ = random effect of period l, l = 1 to 3; $e_{ijkl}$ = random error associated with each observation. The degrees of freedom were calculated according to the Satterthwaite method (DDFM = SATTERTH) and the treatment effect was decomposed into 2 orthogonal polynomial contrasts (linear and quadratic). The orthogonal polynomials coefficients were obtained using the IML procedure of SAS because the treatments were not equally spaced. The significance of main effects was declared at $P < 0.05$ and tendency to significance at $P < 0.10$.

## Results

### Nutrient intake and total tract apparent digestibility

In experiment 1, the intakes of OM, NDF and ADF increased linearly ($P < 0.05$) according to the CP increase, but there was lower starch intake ($P < 0.01$). There was a linear increase in DM digestibility ($P = 0.01$) and CP digestibility ($P < 0.01$) coefficients according to CP increase content (Table 3). There was a tendency to increase the DMI when there was an increase of dietary CP ($P = 0.06$), as what was observed with EE too.

In experiment 2, DMI increased in a quadratic behavior according to the RDP content, and the optimum intake was around 100 g RDP/kg DM ($P = 0.04$). There was a linear increase of the starch intake ($P < 0.01$), but reduction of the NDF and ADF at the highest dose of RDP used ($P < 0.05$).

Table 3 – Nutrient intake of dairy cows according to dietary protein content and protein degradability

| Nutrient | g CP/kg DM | | | SEM | P | | g RDP/kg DM | | | SEM | P | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 130 | 160 | 180 | | L | Q | 80 | 100 | 120 | | L | Q |
| | | | | | | *Intake (kg/d)* | | | | | | |
| DM | 18.9 | 20.4 | 21.2 | 0.50 | 0.05 | 0.06 | 19.9 | 20.4 | 19.1 | 0.44 | 0.13 | 0.04 |
| OM | 17.9 | 19.0 | 19.9 | 0.47 | 0.02 | 0.07 | 18.8 | 19.4 | 18.2 | 0.41 | 0.24 | 0.05 |
| Starch | 6.02 | 5.63 | 4.70 | 0.15 | <0.01 | 0.06 | 5.51 | 5.76 | 6.07 | 0.12 | <0.01 | 0.83 |
| NDF | 5.68 | 6.20 | 6.30 | 0.15 | <0.01 | 0.41 | 5.97 | 6.02 | 5.58 | 0.13 | 0.03 | 0.10 |
| EE | 0.45 | 0.55 | 0.55 | 0.01 | 0.07 | 0.32 | 0.60 | 0.62 | 0.56 | 0.01 | 0.24 | <0.01 |
| ADF | 4.20 | 4.54 | 4.63 | 0.11 | <0.01 | 0.05 | 4.70 | 4.72 | 4.37 | 0.10 | 0.01 | 0.11 |
| | | | | | *Coefficients of total tract apparent digestibility* | | | | | | | |
| DM | 0.64 | 0.68 | 0.70 | 0.003 | 0.01 | 0.18 | 0.67 | 0.69 | 0.67 | 0.006 | 0.59 | 0.08 |
| OM | 0.71 | 0.71 | 0.71 | 0.003 | 0.06 | 0.28 | 0.70 | 0.71 | 0.70 | 0.005 | 0.79 | 0.12 |
| CP | 0.80 | 0.80 | 0.83 | 0.006 | <0.01 | 0.80 | 0.84 | 0.83 | 0.82 | 0.006 | 0.39 | 0.93 |
| Starch | 0.98 | 0.97 | 0.98 | 0.001 | 0.21 | 0.62 | 0.97 | 0.97 | 0.97 | 0.001 | 0.65 | 0.24 |
| NDF | 0.40 | 0.39 | 0.42 | 0.009 | 0.34 | 0.16 | 0.39 | 0.42 | 0.34 | 0.013 | 0.75 | 0.01 |
| EE | 0.67 | 0.67 | 0.67 | 0.016 | 0.81 | 0.43 | 0.72 | 0.77 | 0.65 | 0.013 | 0.67 | <0.01 |
| ADF | 0.43 | 0.42 | 0.45 | 0.007 | 0.19 | 0.16 | 0.45 | 0.48 | 0.41 | 0.012 | 0.87 | <0.01 |

DM: dry matter; OM: organic matter; CP: crude protein; RDP: rumen-degradable protein; NDF: neutral detergent fiber; EE: ethereal extract; ADF: acid detergent fiber; L: linear; Q: quadratic; SEM: Standard error mean; *P*: *P* value.

Furthermore, the coefficients of NDF, ADFD and EE were higher when the RDP was around 100 g/kg DM (Table 3).

### Milk production and composition

In experiment 1, MY increased linearly ($P < 0.01$) according to the increase of CP content. Cows fed 180 g CP/kg DM produced 2.67 kg/d more milk than those fed 130 g CP/kg DM. The FCM production also increased linearly ($P < 0.01$) according to the increase of CP content, and cows fed 180 g CP/kg DM produced 3.56 L/d more FCM than those fed with 130 g CP/kg DM. The milk levels of fat ($P = 0.04$), protein ($P = 0.02$), casein ($P < 0.01$) and lactose ($P < 0.01$) increased linearly according to the CP, and cows fed 180 g CP/kg DM increased 8.3% more casein than cows fed 130 g CP/kg of DM. Similarly, milk total solids increased linearly according to the CP content increase of 130 to 160 g CP/kg DM (11.91 *vs* 12.23 g/100g) ($P = 0.01$). With the increase of CP, the production of milk urea nitrogen (MUN) ($P < 0.01$), fat ($P < 0.01$), protein ($P < 0.01$), casein ($P < 0.01$) and total solids ($P < 0.01$) increased linearly.

In experiment 2, the MY had a quadratic effect according to dietary RDP content. Cows fed an intermediate RDP content (100 g RDP/kg DM) produced 2.54 kg/d more milk than when fed a high RDP (120g RDP/kg DM). Similarly, the FCM ($P < 0.01$), protein content ($P < 0.01$) and casein ($P < 0.01$) had the highest production when cows were fed 100 g RDP/kg DM. Lactose content decreased linearly ($P = 0.03$) from 4.42 to 4.37 g/100g with the increased RDP from 80 to 120 g RDP/kg DM while fat content was not altered by the RDP content ($P > 0.05$). The daily production of fat ($P = 0.03$), protein ($P < 0.01$), casein ($P < 0.01$), lactose ($P < 0.01$) and total solids ($P < 0.01$) had a quadratic effect according to the RDP content, with higher yields occurring around 100 g RDP/kg DM (Table 4).

### Ruminal pH, SCFA and N-NH₃ concentration

In experiment 1, the concentration of $N-NH_3$ increased linearly with the increase of CP content and cows that were fed 180 g CP/kg DM had a $N-NH_3$ ruminal concentration 2.17 times higher than when fed with 130 g CP/kg DM. The concentrations of acetic acid ($P = 0.08$) and propionic acid ($P = 0.09$) tended to have a linear effect according to the CP content. The remaining SCFA and ruminal pH were not altered by the CP content.

In experiment 2, the ruminal pH was reduced in a quadratic manner according to the increase in RDP content ($P < 0.01$). The concentration of propionic acid had a quadratic effect and increased by approximately 4 mmol/L from 80 g RDP for 100g PDR/kg DM ($P = 0.03$). The concentration of acetic acid tends to have a linear effect with the increase of RDP content ($P = 0.05$) and the same linear effect occurs with the concentrations of iso-butyric and valeric acids. The remaining SCFA was not altered by the variation of RDP content. Furthermore, the increase of RDP content led to a linear increase of $N-NH_3$ concentrations of ruminal fluid ($P < 0.01$) (Table 5).

### Nitrogen balance and nitrogen use efficiency for milk production

The nitrogen intake (g/day) increased linearly ($P < 0.01$) according to the increase in CP content in the diet, and

Table 4 – Effect of dietary protein content and protein degradability on milk production and composition of dairy cows

| Variable | g CP/kg DM | | | SEM | P | | g RDP/kg DM | | | SEM | P | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 130 | 160 | 180 | | L | Q | 80 | 100 | 120 | | L | Q |
| | | | | | | *kg/cow/day* | | | | | | |
| MY | 27.6 | 29.8 | 30.3 | 0.66 | <0.01 | 0.13 | 27.5 | 28.0 | 25.4 | 0.54 | <0.01 | <0.01 |
| FCM | 27.9 | 30.6 | 31.4 | 0.77 | <0.01 | 0.20 | 28.3 | 29.4 | 26.2 | 0.87 | 0.02 | <0.01 |
| | | | | | | *g/kg* | | | | | | |
| Fat | 35.3 | 36.7 | 37.1 | 0.6 | 0.04 | 0.75 | 36.6 | 37.4 | 36.4 | 1.1 | 0.91 | 0.52 |
| Protein | 28.2 | 28.7 | 29.0 | 0.3 | 0.02 | 0.83 | 29.3 | 29.8 | 28.2 | 0.3 | <0.01 | <0.01 |
| Lactose | 44.6 | 44.8 | 45.2 | 0.3 | <0.01 | 0.64 | 44.2 | 44.0 | 43.7 | 0.3 | 0.03 | 0.98 |
| TS | 119 | 122 | 122 | 1.1 | 0.01 | 0.10 | 121 | 122 | 119 | 1.5 | 0.36 | 0.36 |
| Casein | 20.8 | 21.7 | 23.2 | 0.2 | <0.01 | 0.04 | 22.2 | 22.5 | 21.3 | 0.3 | <0.01 | <0.01 |
| | | | | | | *mg/dL* | | | | | | |
| MUN | 12.7 | 17.4 | 18.9 | 0.55 | <0.01 | 0.32 | 15.0 | 16.3 | 19.1 | 0.38 | <0.01 | 0.57 |
| | | | | | | *kg/cow/day* | | | | | | |
| Fat | 0.98 | 1.08 | 1.12 | 0.03 | <0.01 | 0.28 | 1.01 | 1.06 | 0.93 | 0.04 | 0.12 | 0.03 |
| Protein | 0.78 | 0.85 | 0.88 | 0.02 | <0.01 | 0.10 | 0.80 | 0.83 | 0.71 | 0.01 | <0.01 | <0.01 |
| Lactose | 1.23 | 1.35 | 1.37 | 0.03 | <0.01 | 0.03 | 1.21 | 1.23 | 1.11 | 0.02 | <0.01 | <0.01 |
| Casein | 0.57 | 0.64 | 0.70 | 0.01 | <0.01 | 0.47 | 0.61 | 0.63 | 0.54 | 0.01 | <0.01 | <0.01 |
| TS | 3.30 | 3.66 | 3.71 | 0.09 | <0.01 | 0.06 | 3.34 | 3.44 | 3.06 | 0.09 | <0.01 | <0.01 |

MY: milk yield; FCM: fat corrected milk for 3.5%; TS: total solids; MUN: milk urea nitrogen; CP: crude protein; DM: dry matter; RDP: rumen-degradable protein; L: linear; Q: quadratic; SEM: Standard error mean; *P*: *P* value.

Table 5 – Adjusted means for the effect of protein content and protein degradability on short-chain fatty acids and ammoniacal nitrogen of dairy cows

| Variables | g CP/kg DM | | | SEM | P | | g RDP/kg DM | | | SEM | P | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 130 | 160 | 180 | | L | Q | 80 | 100 | 120 | | L | Q |
| pH | 6.54 | 6.59 | 6.61 | 0.22 | 0.33 | 0.88 | 6.46 | 6.27 | 6.53 | 0.04 | 0.39 | <0.01 |
| | | | | | | *mmol/L* | | | | | | |
| Acetic acid | 45.4 | 45.9 | 49.4 | 1.15 | 0.08 | 0.45 | 51.5 | 53.0 | 48.2 | 1.31 | 0.05 | 0.52 |
| Propionic acid | 15.4 | 14.7 | 13.7 | 0.44 | 0.09 | 0.90 | 17.0 | 20.5 | 16.4 | 0.97 | 0.74 | 0.03 |
| Butyric acid | 11.7 | 11.2 | 11.6 | 0.35 | 0.84 | 0.52 | 13.0 | 12.7 | 11.4 | 0.54 | 0.15 | 0.56 |
| Isobutyric acid | 0.91 | 0.87 | 0.84 | 0.03 | 0.51 | 0.90 | 1.09 | 0.95 | 0.93 | 0.03 | 0.07 | 0.38 |
| Valeric acid | 1.34 | 1.27 | 1.24 | 0.03 | 0.23 | 0.77 | 1.58 | 1.55 | 1.58 | 0.09 | 0.06 | 0.32 |
| Iso-valeric acid | 1.82 | 1.79 | 1.68 | 0.06 | 0.35 | 0.79 | 2.13 | 2.08 | 2.17 | 0.07 | 0.85 | 0.67 |
| | | | | | | *mg/dL* | | | | | | |
| $NH_3$-N | 12.1 | 18.2 | 26.3 | 1.2 | <0.01 | 0.52 | 11.5 | 18.0 | 27.5 | 1.86 | <0.01 | 0.56 |

CP: crude protein; DM: dry matter; $NH_3$-N: ammoniacal nitrogen; CP: crude protein; RDP: rumen-degradable protein; L: linear; Q: quadratic; SEM: Standard error mean; *P*: *P* value.

cows fed 180 g CP/kg DM consumed 200 g more N of than animals fed 130 g CP/kg DM. Similarly, excretions of nitrogen in feces (*P* = 0.03), urine (*P* <0.01) and milk (*P* <0.01) increased linearly according to the CP content. However, excretion of N in feces and milk (percentage of ingested N) were linearly reduced with increasing CP content, whereas excretion of N in urine (percentage of ingested N) showed a quadratic effect (*P* = 0.02). The N balance (retention; g/d) increased linearly with the increase of CP content (*P* <0.01).

In experiment 2, the nitrogen intake had a quadratic effect according to the increase of RDP content, and the lowest intake was observed with 120 g RDP/kg DM. The increase of RDP content linearly increased the excretion of NU (*P* = 0.04). The excretion of NM showed a quadratic effect, with the lowest excretion of NM observed with the inclusion of 120 g RDP/kg DM (*P* <0.01). The balance of N had a quadratic effect observed (*P* = 0.02) according to the RDP content and the lowest balance was observed with the inclusion of 120 g RDP/kg DM. The excretion of nitrogen in feces tended to have a linear decrease with the increase of RDP (P = 0.05). The protein excretion in milk (percentage of ingested N) increased in a quadratic manner according to the RDP content (*P* <0.01), with the lowest excretion observed with 120 g RDP/kg DM. The excretion of nitrogen in feces decreased linearly with increasing RDP content (*P* = 0.03), while the excretion of nitrogen in urine increased linearly (*P* <0.01), both like a percentage of ingested N (Table 6).

## Discussion

In the present study, there was only a trend to a DMI increase as CP content of the diet increased. Similarly, no effects on DMI were observed in dairy cows of 20 kg milk/day and 110 DIM when the CP level increased from 11.3 to 14.4% of DM (Pereira et al., 2005). On the other hand, some studies have reported a linear increase in DMI by increasing CP levels in the diet of dairy cows (Colmenero & Broderick, 2006a; Ipharraguerre & Clark, 2005). These differences can be explained by the variation of RDP:RUP ratio and dietary characteristics such as ruminal fiber digestibility and NFC source (Broderick et al., 2009). Similarly to the present study, Kalscheur et al. (2006) did not observe effects on DMI in diets with high RDP content. Otherwise, increasing the RDP:RUP ratio from 60:40 to 65:45 was observed to increase milk yield and dietary N use efficiency (Savari et al., 2018). These authors have associated these effects possibly with the lower microbial protein synthesis and/or lower intestinal digestibility of the source of RUP used and the lower ratio RDP:RUP (soybean meal treated with xylose). Indeed, the reduction of the RDP:RUP ratio from 60:40 to 50:50 limited the microbial protein synthesis, but did not alter the productive performance of cows (Savari et al., 2018). Thus, the effects of the RDP:RUP ratio on MY depend on the relationship between how they are used and the sources used as RDP and RUP.

In the present study, the increase of DMI due to increased RDP may indicate that diets with 80 g RDP/kg DM may have limited the microbial protein synthesis and ruminal fermentability of the fiber, while the inclusion of 100 g RDP/kg DM may have optimized ruminal metabolism, explained by the increase on NDF digestibility. However, increasing the RDP inclusion from 100 to 120 g RDP/kg DM did not increase ruminal fermentation and microbial activity and decreased DMI. This decrease can be explained by the lowest value of NDF digestibility (0.34), which increased the digestive tract time and inhibited the DMI. Moreover, the metabolic effects and low palatability of urea may have contributed to lower CMS, although the diet was provided as a TMR. Huber & Cook (1972) suggested that the reduction of DMI in response to urea inclusion is due to low palatability and not because of ruminal or post-ruminal effects. However, cows cannot dislike the flavor of urea per se, but they could identify different levels of urea in rations, and develop an aversion when it is fed at higher levels (Kertz, 2010).

The linear increase of MY, FCM and milk solids production according to the increase of CP content in the present study may be associated with greater DM and CP intake. Similarly, the increases of MY, FCM and total solids according to the RDP inclusion can also be associated with DMI since this variable increased with increasing of RDP content. Broderick (2003) also observed increased MY, FCM and milk protein according to the increase in CP content of the diet (15.1, 16.7 and 18.4%). Pereira et al. (2005) observed a linear increase in the FCM, fat and protein production per day in cows in the middle third of lactation when fed diets with increasing CP levels (11.3, 12.3, 13.3 and 14.4%). The results of the present study, as well as those of Broderick (2003), confirm the hypothesis that the increase of CP content increases availability of metabolizable protein and amino acids for use by the mammary gland, beyond the increase of glucose synthesis from non-used AAs.

In the present study, the linear increase of apparent total digestibility of CP according to the increase in CP content may be related to the effect of fecal metabolic nitrogen dilution (Broderick, 2003; Colmenero & Broderick, 2006b; Pereira et al., 2005). In some studies, increased digestibility of DM, OM, CP and NDF was observed with increasing CP content of the diet (Broderick, 2003; Colmenero & Broderick, 2006a; Pereira et al., 2005). There was greater apparent digestibility of DM and CP as CP concentration in the diet increased, indicating that the rumen environment benefited, resulting in greater intake and MY. The lack of effect on NDF is totally expected once the ruminal passage rate increases with intake (Dado & Allen, 1995), diminishing the time that fiber stays in the rumen to be digested.

Urinary N excretion was greater for cows fed higher CP and RDP content, suggesting that these diets produced higher amounts of $N-NH_3$ in the rumen, which was observed in both experiments. The absorption of $N-NH_3$ by the ruminal epithelium resulted in increased production of blood urea via the urea cycle. Thus, when cows are fed higher levels of CP or RDP, there is a greater excretion of urinary N to eliminate blood urea N. In a review about the efficiency of nitrogen use in diets of dairy cows, Castillo et al. (2000) reported that when N ingestion was greater than 400 g N/d, the N urinary excretion increased exponentially, but the rate of N excretion in feces decreased linearly, as we observed in this study with the RDP increase. This fact emphasizes the importance of the dietary N balance, as increasing CP or RDP increases N elimination by milk and urine. These authors suggested that the use of 150 g of CP/kg DM would be an optimal value to reduce the excretion of urinary N, without altering MY. However, these results should be interpreted with caution, since the RDP:RUP ratio, metabolizable protein content and amino acid profile can also determine the optimum CP

content to optimize productive performance and reduce N excretion (Broderick et al., 2009). The results of experiment 1 indicated that inclusion of RUP up to 100 g/kg optimized milk protein synthesis, and inclusions above this content resulted only in increased urine N excretion and MUN. Increasing urinary N excretion and MUN, and reducing fecal N excretion, is due to the higher amount of NNP in the rumen-absorbed diet and the lower amount of RUP, which is intended for intestinal absorption.

The reduction of CP content from 15.4 to 12.4% of DM reduced urinary N excretion and did not alter milk protein content (Agle et al., 2010). Different from our study, Savari et al. (2018) observed greater efficiency of N use by increasing the RDP:RUP ratio from 60:40 to 65:45, due to greater N secretion in milk. In the present study, there was an effect of CP and RDP levels on the feces N excretion (g/d). Reduction of fecal N was observed for diets with higher inclusion of RDP, since the available N in the rumen could be used by the ruminal microbiota or be absorbed as $N-NH_3$ and eliminated in urine and milk, resulting in a lower N flux to the gut and fecal excretion. Mendonça et al. (2004) did not observe an increase in the excretion of N in feces with urea inclusion (0.35 or 1% DM) in the diet. In another study, the increase in CP content of the diet also had no effect on fecal N excretion (Agle et al., 2010).

In the present study, there was a greater amount of nitrogen secreted by milk as a proportion to total N intake of cows fed diets with low CP, which resulted in a higher efficiency of N ingestion for conversion to milk protein, but leading to reduction of the MY. Previous studies have also observed a higher efficiency of dietary N utilization after a reduction of total dietary N (Broderick & Reynal, 2009; Colmenero & Broderick, 2006a; Lee et al., 2012).

We observed that an increase of CP levels from 130 to 180 g CP/kg DM increased DMI and performance of dairy cows, but there was a reduction in the efficiency of dietary N utilization. Inclusion of 100 g RDP/kg DM optimized DMI and performance, but urine N elimination increased linearly with RDP inclusion from 80 to 120 g RDP/kg DM. Therefore, it is advisable that the increase of CP through increased RDP should be up to values close to 100 g RDP/kg of DM, and values above this should be supplied using high quality rumen bypass protein to optimize performance production and N use efficiency of dairy cows.

## Conclusion

The CP levels from 130 to 180 g CP/kg DM, through increased RDP levels to values close to 100 g RDP/kg of DM, increased DMI and performance of dairy cows with an increase of ammoniacal nitrogen. However, this increase of protein availability can lead to a reduction in the efficiency of dietary N utilization.

## Conflict of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

## Ethics Statement

This study was approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science (CEUA/FMVZ/USP - protocol number 2671/2012).

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## Abbreviations

CP, crude protein; DM, dry matter; RDP, rumen-degradable protein; DMI, dry matter intake; MY, milk yield; NDF, neutral detergent fiber; ADF, acid detergent fiber; FCM, fat corrected milk; NH3-N, ammoniacal nitrogen; SCFA, short-chain fatty acids.