Livestock Science 187 (2016) 35–39

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Short communication

Dose response of chitosan on nutrient digestibility, blood metabolites and lactation performance in holstein dairy cows

R.D. Mingoti [a], J.E. Freitas Jr.[a], J.R. Gandra [a], R. Gardinal [a], G.D. Calomeni [a], R.V. Barletta [a], T.H.A. Vendramini [a], P.G. Paiva [b], F.P. Rennó [a,*]

[a] Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo (USP), Av. Duque de Caxias Norte, 225-Campus da USP, 13635-900 Pirassununga, SP, Brazil
[b] Department of Animal Science, UNESP – Universidade Estadual Paulista "Júlio de Mesquita Filho"/Campus Jaboticabal, Rod. Prof. Paulo Donato Castellane km 5, Rural, 14884-900 Jaboticabal, SP, Brazil

ARTICLE INFO

Article history:
Received 9 June 2015
Received in revised form
23 February 2016
Accepted 25 February 2016

Keywords:
Chitosan
Digestibility
Intake
Dairy cow

ABSTRACT

Chitosan (CHI), a non-toxic and biodegradable biopolymer has been successfully used in the food, human and veterinary medicine industries, especially because of its antimicrobial properties. This study aimed to investigate the effects of CHI on dry matter intake (DMI), nutrient digestibility, blood metabolites, milk yield and composition, and milk fatty acids profile of lactating dairy cows. Sixteen Holstein cows (91.3 ± 15.1 days in milk, 29.8 ± 4.1 kg/d of milk, 610 ± 55 kg of body weight [BW]) were used in a 4 × 4 Latin square design, and each experimental period consisted of 14 days of diet adaptation and 7 days for data collection. Animals were allocated to receive one of treatments: C0: basal diet, without CHI addition; C50, C100 and C150 received 50, 100 and 150 mg/kg BW of CHI, respectively. Chitosan addition did not affect DMI, but increased ($P \leq 0.05$) the digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and neutral detergent fiber (NDF). Blood urea nitrogen concentration was increased ($P=0.01$) with CHI addition. Chitosan decreased ($P=0.02$) nitrogen (N) fecal excretion without affect nitrogen balance. There was no effect of CHI on milk yield, fat corrected milk, and milk composition. Chitosan decreased ($P \leq 0.05$) C6:0 and C18:1 c9, and quadratically affected C14:1 fatty acids (FA) concentration in milk fat. Chitosan had no effect on total saturated and unsaturated FA of milk fat. Chitosan improved nutrient digestibility without affect productive performance and milk fatty acid profile of mid-lactation dairy cows.

1. Introduction

Currently, possible bacterial resistance with the use of antibiotics as additive to improve animal performance led to increase of the interest of scientific and industry related to the use of natural additives compounds in food preservation, human and animal health (Gois et al., 2016).

Chitosan (CHI) is a nontoxic and biodegradable biopolymer that has received attention due to its potential application in human and veterinary medicine and food preservation, notably for its properties against bacteria, fungi and yeasts (Kong et al., 2010). However, the use of CHI as an animal feed additive has been poorly explored, with few studies in non-ruminant digestion aiming to improve nitrogen retention, feed efficiency, and performance (Huang et al., 2005; Xu et al., 2013).

In ruminant nutrition, several studies had showed that CHI could change ruminal fermentation, including higher propionate concentration and lower acetate to propionate ratio, likely improving the energy efficiency of ruminal fermentation (Goiri et al., 2009a; Goiri et al., 2010a). However, *in vitro* studies reported that CHI decreased dry matter (DM) digestibility, especially in high fiber mixtures in batch cultures (Goiri et al., 2009a; Goiri et al. 2009b). On the other hand, CHI was effective to inhibit biohydrogenation by increasing C18:1, *t*11 fatty acid (FA) and total conjugated linoleic acid (CLA) proportions and decreasing the saturated FA proportion an *in vitro* conditions (Goiri et al., 2010b); and currently, there are few studies evaluating the effects of CHI *in vivo*. Therefore, the objective of this study was to evaluate the effects of CHI on dry matter intake (DMI), nutrient digestibility, blood metabolites, nitrogen balance, milk yield and composition, and milk fatty acids profile of lactating dairy cows.

* Corresponding author.
E-mail address: francisco.renno@usp.br (F.P. Rennó).

http://dx.doi.org/10.1016/j.livsci.2016.02.008
1871-1413/

2. Materials and methods

The Ethics Committee of the School of Veterinary Medicine and Animal Science of the University of Sao Paulo, approved the experimental procedures (approval number 2222/2011).

2.1. Animals, design and treatments

Sixteen Holstein cows were used that averaged 91.3 ± 15.1 days in milk, 610 ± 55 kg of BW; 29.8 ± 4.1 kg/d of milk yield at the beginning of the trial. Cows were housed in individual pens with 17.5 m^2, sand beds and forced ventilation. Each experimental period consisted of 14 days of diet adaptation and 7 days for data collection. The cows were allocated into a replicate 4 × 4 Latin square design to receive one of four treatments: C0: basal diet, without chitosan addition; C50, C100 and C150 received 50, 100 and 150 mg/kg BW chitosan, respectively. Chitosan was provided daily to cows into two equal portions, weighed and top dressed to the total mixed ration before the morning and afternoon feeding, and cows were fed twice daily (0700 h and 1300 h) to maintain refusals between 5% and 10% (on as fed basis). Chitosan used in this study had 0.33 g/mL of apparent density, pH of 8.81, and deacetylation degree of 86.6% (Polymar® Science and Nutrition, Fortaleza, Ceara, Brazil). Basal control diet was formulated to meet requirement of lactating dairy cows producing 30.0 kg/d of milk yield with 3.5% of fat according to the recommendations of NRC (2001). Basal control diet consisted of 50% of corn silage, 23.73% of ground corn, 14.50% of soybean meal, 8.01% of whole raw soybeans, 0.35% of urea, 0.10% of ammonium sulfate, 0.80% of sodium bicarbonate, 0.09% of magnesium oxide, 0.14% of limestone, 0.24% of salt, and 1.98% of mineral premix (190 g/kg of Ca, 73 g/kg of P, 44 g/kg of Mg, 30 g/kg of S, 340 mg/kg of Cu; 1350 mg/kg of Zn, 940 mg/kg of Mn, 16 mg/kg of I, 3 mg/kg of Co, 100 mg/kg of Se, 1064 mg/kg of Fe, 10,000 UI of vitamin-A, 4000 UI of vitamin-D, and 6000 UI of vitamin-E) on DM basis (Supplementary data). Throughout the experiment, BW and body condition score (BCS) were measured at the first and day 21 of each period after the morning milking.

2.2. Data and sample collection

Samples of feed and orts were collected throughout the sampling period and stored at −20 °C until analyses. During days 16–18 of each experimental period, fecal samples were collected from each cow, after a.m. and p.m. milking, comprising a composite sample per cow. Samples of feeds, orts and feces were dried in a 65 °C forced-air oven for 72 h, ground to pass through a 1 mm screen (Thomas Wiley Mills, Thomas Scientific, Swedesboro, USA) and analyzed for dry matter (DM) (method 930.15; Association of official Analytical Chemists, 2000), crude protein (CP) (N × 6.25; method 984.13; Association of official Analytical Chemists, 2000), ether extract (EE) (method 920.39; Association of official Analytical Chemists, 2000), acid detergent fiber (ADF) (method 973.18; Association of official Analytical Chemists, 2000), ash (method 942.05; Association of official Analytical Chemists, 2000). Neutral detergent fiber (NDF) was analyzed according to Mertens (2002), using thermo-stable alpha-amylase without addition of sodium sulfite to detergent (Ankom Technology, Macedon, USA).

Indigestible acid-detergent fiber (iADF) was used as an internal marker to estimate fecal excretion and apparent total-tract digestibility of nutrients. Dried samples of feeds, refusals and feces were processed in a Wiley mill through a 2 mm screen (Thomas Wiley Mills, Thomas Scientific, Swedesboro, USA). These samples were placed in bags of non-woven textile (100 g m^{-2}) following the recommendation of a maximum of 20 mg of DM/cm^2 (Nocek, 1988), and then incubated for 288 h in the rumen of two Holstein cows previously adapted to a similar diet used in this study according to Casali et al. (2008). After the removal from rumen, bags were washed in running tap water, dried at 65 °C in a forced-air oven and submitted to acid-detergent solution in an Ankom® System (Ankom Technology, Macedon, USA) to obtain the iADF concentrations as previously described.

2.3. Milk production, composition and fatty acid profile

Cows were mechanically milked twice daily at 0600 and 1600 h, and milk production was measured by an automatic milk meter (Alpro®, DeLaval – Tumba, Sweden). Milk samples were collected from each cow on days 16, 17 and 18 of the experimental period and then analyzed for CP, fat, and lactose (Milkoscan; Foss Eletric, Hillerod – Denmark). Milk yield were corrected for 3.5% of fat according to Sklan et al. (1992).

Milk FA extraction was performed according to Feng et al. (2004) and separated fat was methylated according to Kramer et al. (1997). Fatty acids were quantified by gas chromatography (GC Shimatzu 2010, Shimadzu Corporation, Kyoto – Japan) using an SP-2560 capillary column (100 m × 0.25 mm i.d. with 0.02-μm film thickness; Supelco Sigma-Aldrich Group, Bellefonte, Pennsylvania, USA). The oven temperature was 70 °C for 4 min, increased 13 °C/min to 175 °C, and then held at this temperature for 27 min. Finally, temperature was increased by 4 °C/min until reached 215 °C, and kept for 31 min. Hydrogen was used as the carrier gas flowing at 40 cm^3/s. Four standards were used for FA identification: standard C4–C24 FA (TM 37; Supelco Sigma-Aldrich Group, Bellefonte, Pennsylvania, USA), C18:1 trans-11 FA (V038-1G; Supelco Sigma-Aldrich Group, Bellefonte, Pennsylvania, USA), C18:2 trans-10, cis-12 FA (UC-61M 100 mg; NU-CHEKPREP, Inc. Elysian, Minnesota, USA), and C18:2 cis-9, trans-11 FA (UC-60M 100 mg; NU-CHEKPREP, Inc. Elysian, Minnesota, USA).

2.4. Nitrogen balance, microbial protein synthesis and blood metabolites

The daily urine volume was estimated using the creatinine concentration (mg/L) in spot urine sample, obtained four hours after the morning feeding on days 16 and 17. Creatinine concentrations were analyzed using a biochemical colorimetric kit (kinetic creatinine: cat. no. K-067, Bioclin, Belo Horizonte, Brazil) and a semiautomatic spectrophotometer (SBA 200, CELM, São Caetano do Sul, Brazil). A daily creatinine excretion rate of 24.05 mg/kg of BW was assumed (Chizzotti et al., 2008). The total excretion of uric acid (uric acid stable liquid: cat. no. K-052, Bioclin, Belo Horizonte, Brazil; determined in a semi-automatic spectrophotometer SBA 200, CELM, São Caetano do Sul, Brazil). The excretion of uric acid, allantoin in the urine and milk were considered as the total excretion of purine derivatives, and microbial protein synthesis was estimated from these concentrations according to Chen and Gomes (1992). The total nitrogen in urine samples was determined (method 984.13; Association of official Analytical Chemists, 2000) and the N balance was determined using the model of NRC (2001).

Blood samples were collected on the day 15 by puncture of the coccygeal vein, before the morning feeding. Serum was obtained by centrifugation of the samples at 3000*g* for 10 min. The blood urea nitrogen (BUN) was analyzed with commercially colorimetric kits (urea: cat. no. K-056; Bioclin®, Belo Horizonte, Brazil), and reading was performed using a semi-automatic spectrophotometer (SBA 200, CELM, São Caetano do Sul, Brazil).

2.5. Statistical analysis

Data were analyzed with PROC MIXED (Statistical Analysis System for Windows 9.3, SAS Institute Inc., Cary, USA), according