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Increasing doses of chitosan to grazing beef steers: Nutrient intake and digestibility, ruminal fermentation, and nitrogen utilization

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ABSTRACT

Chitosan (CHI) is a derivative of the biopolymer chitin, found in high amounts in the shell wastes of crustaceans, and has antimicrobial properties. The objective of this study was to determine the influence of increasing doses of CHI for grazing cattle on nutrient intake and total apparent digestibility, ruminal fermentation parameters, microbial protein synthesis, nitrogen utilization, and urea and creatinine metabolism. Five rumen cannulated crossbred steers [3.6 mo and 300 ± 25 kg live weight (LW), mean ± SD] were used in a 5 × 5 Latin square experiment design with 21-d periods, in which the last 7 days were used for data collection. Steers were randomly designated to one treatment sequence containing chitosan (≥850 g/kg deacetylation degree, 0.32 g/mL density, pH 7.90, and viscosity <200cPs) added at 0, 400, 800, 1200 or 1600 mg/kg DM of concentrate. Animals were individually allocated in paddocks (0.3 ha) uniformly covered with *Urochloa brizantha* and were fed a concentrate at 150 g/100 kg of LW containing 300 g/kg of crude protein (CP, on DM basis). Chitosan quadratically affected ($P \leq 0.007$) forage and neutral detergent fiber intake, in which the highest values were observed at intermediate doses of CHI. Chitosan linearly increased ($P \leq 0.034$) the DM and CP total apparent digestibility of steers. Chitosan had no effect on ruminal pH, and quadratically influenced ($P = 0.005$) ruminal ammonia nitrogen concentration. Chitosan linearly increased ($P = 0.007$) ruminal propionate concentration, without affecting the total short-chain fatty acid concentration. Furthermore, CHI quadratically affected ($P = 0.044$) the acetate to propionate ratio in rumen. Treatments quadratically affected ($P = 0.032$) microbial protein synthesis, in which the highest value was estimated when supplying CHI at 800 mg/kg DM of supplement. Nitrogen intake was quadratically affected ($P = 0.006$) by treatments. Chitosan neither affected urinary nor blood concentrations of urea and creatinine. However, CHI quadratically affected ($P = 0.002$) the urea excretion of steers. In conclusion, CHI increased DM intake and digestibility, as well as propionate concentration and calculated microbial CP production in steers.

Abbreviations: ADF, acid detergent fiber; CHI, chitosan; CP, crude protein; DM, dry matter; EE, ether extract; $_{i}$NDF, indigestible neutral detergent fiber; LW, live weight; NDF, neutral detergent fiber; P_{abs}, absorbed purines; $_{pd}$DM, potentially digestible dry matter; PD, purine derivatives; SCFA, short-chain fatty acids.

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1. Introduction

Chitosan (CHI) is formed from the deacetylation of chitin (a by-product of fishing industry, especially from shrimp, lobster, krill, and crab), the second most abundant biopolymer in nature. In addition, CHI is non-toxic, biodegradable and has been identified as safe for use in food by the US Food and Drug Administration (2012). Several CHI applications were reviewed by Senel and Mcclure, (2004); recently, due to its antimicrobial activity, CHI has also been applied as silage inoculant (Gandra et al., 2016a) and supplied as rumen modulator either to confined beef steers (Araújo et al., 2015) or to lactating dairy cows (Paiva et al., 2016) with promising results.

Although CHI antimicrobial mechanism is not fully elucidated, the intracellular leakage mechanism is the most accepted theory by scientific community (Helander et al., 2001; Kong et al., 2010). In this theory, positively charged CHI binds to bacterial negatively charged surface, altering membrane permeability (hydrolysis of peptidoglycans), resulting in leakage of intracellular components and consequently cell death (Helander et al., 2001). Henry et al. (2015) evaluated CHI provision to beef heifers receiving a most-forage diet and reported improvements on neutral detergent fiber (NDF), acid detergent fiber (ADF) and DM digestibility. These authors assessing the effects of CHI on *in vitro* batch cultures also described greater total volatile fatty acid production for batches with CHI compared to those with monensin. Moreover, Belanche et al. (2016) in a rumen simulation study stressed that CHI shifted the fermentation pattern from acetate towards propionate production.

Thus, it is expected that CHI supplementation should benefit fiber digestibility, pasture intake, and ruminal fermentation in grazing cattle. In addition, the speed of concentrate intake of grazing cattle may alter ruminal fermentation by rapidly decreasing rumen pH and consequently impairing ruminal fermentation. Goiri et al. (2009) evaluated increasing levels of different CHI through *in vitro* trials and reported that CHI increased pH in fermentation batches containing forage to concentrate mixture of 80:20. This study was carried out to determine the influence of increasing doses of CHI on nutrient intake and total apparent digestibility, ruminal fermentation, microbial protein synthesis, nitrogen utilization, and urea and creatinine metabolism of grazing beef steers.

2. Material and methods

The current experiment agrees with the principles established by the Ethics Committee from Federal University of Grande Dourados (approval protocol: 023/2015 CEUA/UFGD). This study was performed between July and September at the Ruminant Nutrition facility and Animal Nutrition Laboratory from the School of Agrarian Sciences of Federal University of Grande Dourados, Dourados, Brazil.

2.1. Animals, paddocks and treatment

Five rumen cannulated crossbred steers [3.6 mo and 300 ± 25 kg live weight (LW), mean ± SD] were used in a 5 × 5 Latin square experiment design with 21-d periods, in which the last 7 days were used for data collection. Steers were randomly designated to one treatment sequence: chitosan (≥850 g/kg deacetylation degree, 0.32 g/mL density, pH 7.90, viscosity <200cPs, total ash 1.35 g/100 g, and loss on drying 9.3 g/100 g; Polymar, Fortaleza, Brazil) added at 0, 400, 800, 1200 or 1600 mg/kg DM of concentrate. Treatment sequence was determined to diminish carry-over effects according to Kaps and Lamberson (2009). Animals were individually allocated in paddocks (0.3 ha) uniformly covered with *Urochloa brizantha* (syn. *Brachiaria brizantha*) and were fed concentrate at 150 g/100 kg of LW. Concentrate consisted of ground corn (435 g/kg DM), soybean meal (40.0 g/kg DM), urea (85 g/kg DM), and a mineral mixture (440 g/kg DM). Mineral mixture contained per kg of product 380 g CP (minimum), 323 g protein equivalent from non-protein nitrogen (max.), 20 g P, 50 g S, 74 g Na, 7.5 mg Co, 147.2 mg Mn, 1.8 mg Se, 525 mg Zn, 200 mg F (max.). Animals were weighed at the last day of each experimental period to adjust concentrate supply. Diets were formulated according to NRC (1996) to maintenance level.

2.2. Pasture availability and chemical composition

The available pasture was determined at the first day of each period by cutting forage at ground level from 10 areas (0.25 m^2) in each paddock. Samples were individually weighed and composited in one sample from each paddock. Then, the composited samples were analyzed for morphological characteristics (Table 1). The ingested forage (extrusa) collection was also performed at the first day of each period after ruminal evacuation as described by Dubbs et al. (2003). Before the extrusa collection, animals were submitted to 12 h fasting to ensure total forage intake, then animals were allocated in the paddocks. After 30 min grazing, rumen was evacuated and a subsample of digesta (400 g) was frozen for chemical composition analysis.

Samples of extrusa and concentrate were analyzed for DM (method 930.15), CP (N × 6.25; method 984.13) and ether extract (EE; method 920.39) according to AOAC (2000). Ash was determined after 4 h at 600 °C in a muffle furnace. Neutral detergent fiber and ADF were determined in a fiber analyzer (TE-149 fiber analyzer, Tecnal Equipment for Laboratory Inc., Piracicaba, Brazil) using alpha amylase and no sodium sulfide (Van Soest et al., 1991). Potentially digestible DM ($_{pd}$DM) was estimated according to (Paulino et al., 2008). Samples of extrusa and concentrate were ground (2-mm particle size), placed in non-woven textile bags (5 × 5 cm, 20 mg DM/cm^2) and incubated in the rumen of two crossbred steers adapted to a similar diet of this experiment. After 288 h, bags were removed from the rumen, washed in running tap water and analyzed for