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Effects of chitosan on ruminal fermentation, nutrient digestibility, and milk yield and composition of dairy cows

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Abstract. Our objective was to evaluate the effects of providing increasing levels of chitosan on nutrient digestibility, ruminal fermentation, blood parameters, nitrogen utilisation, microbial protein synthesis, and milk yield and composition of lactating dairy cows. Eight rumen-fistulated Holstein cows [average days in lactation = 215 ± 60.9; and average bodyweight (BW) = 641 ± 41.1 kg] were assigned into a replicated 4 × 4 Latin square design, with 21-day evaluation periods. Cows were assigned to be provided with four levels of chitosan, placed into the rumen through the fistula, as follows: (1) Control: with no provision of chitosan; (2) 75 mg/kg BW; (3) 150 mg/kg BW; and (4) 225 mg/kg BW. Chitosan had no effect on dry matter intake ($P > 0.73$); however, chitosan increased ($P = 0.05$) crude protein digestibility. Propionate concentration was increased ($P = 0.02$), and butyrate, isobutyrate, isovalerate and acetate : propionate ratio were decreased ($P \leq 0.04$) by chitosan. Chitosan had no effect ($P > 0.25$) on acetate, pH and NH_3 ruminal concentration. Glucose, urea, and hepatic enzyme concentrations in the blood were similar ($P > 0.30$) among treatments. Nitrogen balance was not affected, but chitosan increased milk nitrogen ($P = 0.02$). Microbial protein synthesis was not affected by chitosan ($P > 0.44$). Chitosan increased ($P = 0.02$) milk yield, fat-corrected milk, protein and lactose production. Chitosan changes ruminal fermentation and improves milk yield of lactating dairy cows; therefore, we conclude that chitosan can be used as a rumen modulator instead of ionophores in diets for dairy cows.

Additional keywords: additive, antimicrobial, dry matter intake.

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Introduction

Meeting requirements of high-production dairy cows is a great challenge for nutritionists. Modulating ruminal fermentation using feed additives is a reality (Calsamiglia *et al*. 2007; Goiri *et al*. 2010), and ionophores are frequently used to improve ruminant nutrient utilisation. Despite the fact that ionophores are the major class of additive used in diets, their use has been reviewed and restricted due to their potential effect on microbial resistance to antibiotics (Russell and Houlihan 2003).

Chitosan, a non-toxic and biodegradable biopolymer derived from the deacetylation of chitin, has been successfully used in the food, pharmaceutical, cosmetics and agricultural industries, especially because of its antimicrobial properties (Kong *et al*. 2010). In ruminant nutrition, previous *in vitro* studies showed that chitosan could change ruminal fermentation by shifting the volatile fatty acid (VFA) profile and increasing propionate concentration (Goiri *et al*. 2009*a*, 2009*b*). However, these studies also reported that chitosan had a negative effect on dry matter (DM) and neutral detergent fibre (NDF) digestibility yet Goiri *et al*. (2010) demonstrated that chitosan increased ruminal propionate concentration without any effect on digestibility in sheep and shifted ruminal fermentation to more efficient routes. Thus, given the importance of ruminal fermentation on metabolism and performance of ruminants, and possible use of chitosan as an alternative to ionophores, more *in vivo* studies are necessary to identify the effect of chitosan, mainly in dairy cow diets. Therefore, our objective was to evaluate the effects of chitosan levels on nutrient intake and digestibility, ruminal fermentation, blood parameters, nitrogen (N) utilisation, microbial protein synthesis and milk yield and composition of late-lactating cows.

Materials and methods

This study was approved by the Bioethics Committee of the School of Veterinary Medicine and Animal Sciences of the University of Sao Paulo (approval number: 3057/2013).

Animals, experimental design and treatments

Eight rumen-fistulated Holstein cows [average 215.4 ± 60.9 days in lactation and average 641.6 ± 41.1 kg of bodyweight (BW)] were assigned into a replicated 4 × 4 Latin square design based on initial milk yield and BW. Each experimental period consisted of 21 days, with 14 days of acclimation to treatments and the last 7 days for data collection. Cows were assigned within each square to be provided with one of four levels of chitosan, as follows: (1) Control: with no provision of chitosan; (2) 75 mg/kg BW; (3) 150 mg/kg BW; and (4) 225 mg/kg BW.

Chitosan used in this study, purchased from a private company (Polymar Indústria, Comércio Importação e Exportação LTDA, Fortaleza, Ceara, Brazil), had 0.33 g/mL of apparent density, pH = 7.9, viscosity <200 cPs, deacetylation degree of 86.3%; 1.4% ash, and 88.3% of DM. The amount of chitosan provided for each cow was daily weighed, packed in paper bags, and placed into the rumen via the fistula, twice daily, at 0800 hours and 1600 hours. Control-based diet was formulated according to NRC (2001; Table 1), and cows were fed a total mixed ration, twice daily at 0700 hours and 1300 hours to provide 105–110% of the expected feed intake. Animals were housed in individual pens of 17.5 m^2 with sand beds and forced ventilation, and had free access to water. Throughout the experimental period, BW was measured in 7-day intervals after the morning milking to allow for adjustment of chitosan provided to each cow.

Table 1. Ingredients and chemical composition of the control-based diet

Item	Control diet
Ingredients (% *DM*)	
Corn silage	63.08
Ground corn	22.52
Soybean meal	11.50
Urea	0.75
Ammonium sulfate	0.15
Mineral premix[A]	1.80
Salt	0.20
Chemical composition (% *DM*)	
Dry matter (% as fed)	52.79
Organic matter	94.07
Crude protein	14.90
Ether extract	2.89
Non-fibrous carbohydrate[B]	38.11
Neutral detergent fibre	39.74
Net energy[C] (Mcal/kg DM)	1.67

[A]Composition (per kg): 88.0 g of Ca; 42.0 g of P; 18.0 g of S; 45.0 g of Mg; 123.0 g of Na; 14.0 mg of Co; 500.0 mg of Cu; 20.0 mg of Cr; 1050.0 mg of Fe; 28.0 mg of I; 1400.0 mg of Mn; 18.0 mg of Se; 2800.0 mg de Zn; 80.0 mg of Biotin; 200.00 000 IU Vit A; 40.00 000 IU Vit D; 1.20 000 IU Vit E.
[B]NFC = 100 – [(CP – CP of urea + urea) + NDF + EE + ash] from Hall (2000).
[C]Estimated using the NRC (2001) model.

Sample collection and analyses

Feed and orts samples were collected throughout the sampling period and stored at −20°C until analysis. From Day 16 to 18 of each period, faecal samples were collected from each cow, after the morning and afternoon milking and combined to make a composite sample per cow. Feed, orts and faecal samples were dried in a forced-air oven at 55°C for 72 h, ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA, USA) and then analysed for DM (method 930.15; AOAC 2000); crude protein (CP) was obtained by multiplying total N, determined using the micro Kjeldahl technique (method 984.13; AOAC 2000), by a fixed conversion factor (6.25); ether extract (EE) was determined gravimetrically after extraction using petroleum ether in a Soxlet apparatus (method 920.39; AOAC 2000), and ash (method 942.05; AOAC 2000). The NDF was determined according to Mertens *et al*. (2002) using fibre determination equipment (Ankom Tech. Corp., Fairport, NY, USA).

Indigestible acid detergent fibre (iADF) was used as an internal marker to estimate total faecal excretion and, consequently, apparent total tract digestibility of nutrient according to Casali *et al*. (2008). Briefly, feed, orts and faeces samples were dried at 55°C in a forced-air oven for 72 h and then ground to pass through a 2-mm screen (Wiley mill, Arthur H. Thomas). The ground material was placed in 20 × 10-cm non-woven textile bags (diameter of pore size = 50 mm and weighing 6 g to provide a sample weight : bag surface ratio of 10–20 mg/cm2; Nocek 1988), and incubated for a 288-h period in the rumen of two Holstein cows, previously adapted to a diet similar to the diet used in this study. After removal, bags were washed in running tap water, then dried at 55°C in a forced-air oven and submitted to an acid-detergent solution treatment to obtain the iADF (973.18; AOAC 2000). Digestibility was calculated using the ratio of iADF in feed (corrected for orts) and faeces.

Ruminal fermentation parameters

Ruminal fluid samples were collected immediately before, and 2, 4, 6, 10 and 12 h after the morning feeding on Day 20 of each period. The ruminal pH values were determined using a digital pH meter (model MB-10, Marte Cientifica, Santa Rita do Sapucai, MG, Brazil). Afterwards, ruminal fluid samples (50 mL) were centrifuged at 7000*g* at 4°C for 15 min. A 2-mL supernatant aliquot was mixed with 0.4 mL of formic acid, and stored at −20°C for analysis of VFA. Another 2-mL supernatant aliquot was mixed with 0.5 mmol/L of sulfuric acid and stored at −20°C for determination of ammonia-N concentration by the phenol-hypochlorite method (Broderick and Kang 1980). Ruminal fluid VFA were measured using a gas chromatograph (model GC-2104, Shimadzu, Tokyo, Japan) according to the method described by Erwin *et al*. (1961) and adapted by Getachew *et al*. (2002). The gas chromatograph was equipped with a split injector and dual flame ionisation detector temperature at 250°C and with a capillary column

(Stabilwax, Restek, Bellefonte, PA, USA) at 145°C. Frozen ruminal fluid samples were thawed at room temperature and centrifuged at 14 500*g* at 4°C for 10 min; then 1 mL of the supernatant subsamples was transferred into a clean dry vial containing 100 μL of internal standard (2-ethylbutyric acid 100 mM; Chem Service Inc., West Chester, PA, USA). The gases used in the analyses were helium as the carrier gas (8.01 mL/min flow), hydrogen as the fuel gas (pressure of 60 kPa), and synthetic air as the oxidiser gas (pressure of 40 kPa). External standard was prepared with acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids (Chem Service). The software GCSolution (Shimadzu) was used for calculation of VFA concentrations.

Blood metabolites

On Day 15 of each period, blood samples were collected from each cow via the coccygeal vein before the morning feeding. Blood samples were centrifuged at 3000*g* at 4°C for 10 min and plasma was separated and stored at −20°C until analysis. Analyses were performed using colourimetric commercial kits (glucose: cat. no. K-082; urea: cat. no. K-056; aspartate-succinyltransferase: cat. no. K-048; gamma-glutamyl transferase: cat. no. K-080; Bioclin, Belo Horizonte, MG, Brazil), and measurements were completed in a semi-automatic spectrophotometer (model SBA 200, CELM, Sao Caetano do Sul, SP, Brazil).

Nitrogen balance and microbial protein synthesis

On Day 16 of each period, spot urine samples were collected from each cow 4 h after the morning feeding. Urine samples were filtered and 10-mL aliquots were immediately diluted in 40 mL of 0.036 *N* sulfuric acid, and stored at −20°C for uric acid and allantoin analyses. A pure urine sample was stored for total N and creatinine analyses. Concentrations of uric acid and creatinine were measured by using biochemical commercial kits (uric acid stable liquid: cat. no. K-0.52; kinetic creatinine: cat. no. K-067; Bioclin), in a semi-automatic spectrophotometer (model SBA 200, CELM). The daily urine volume was estimated from the daily creatinine as 24.05 mg/kg of BW (Chizzotti *et al*. 2008). The excretion of uric acid and allantoin in the urine and milk (Fujihara and Yamaguchi 1978), were considered as the total excretion of purine derivatives, and microbial protein synthesis was estimated from these concentrations according to Chen and Gomes (1992). Total N in urine samples was determined (method 984.13; AOAC 2000), and N balance was calculated according to the NRC (2001) model.

Milk yield and composition

Cows were milked twice daily, at 0600 hours and 1600 hours, and milk yield was measured with an automatic milk meter (model Alpro, DeLaval, Tumba, Sweden). From Day 16 to 18 of each period, milk samples were automatically collected (model Alpro), according to the milk yield of each cow and of each milking. Fresh milk samples were analysed for CP, fat, and lactose using an ultrasonic milk analyser (model MCC, Milkotronic Ltd, Nova Zagora, Bulgaria). Milk yield was corrected to 3.5% fat according to Sklan *et al*. (1992).

Statistical analyses

Data were analysed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA, version 9.0), according to the statistical model:

$$Y_{ijk} = \mu + S_i + P_j + T_k + A_l(S_i) + e_{ijk},$$

where Y_{ijk} = dependent variable, μ = overall mean, S_i = fixed effect of square, P_j = fixed effect of period, T_k = fixed effect of treatment, A_l (S_i) random effect of animal within square and e_{ijk} = residual error. Ruminal fermentation variables (pH, NH_3-N, and VFA) were analysed as repeated using the MIXED procedure of SAS. The statistical model included the effects of animal, period, square, treatment, time and time*treatment interaction. Compound symmetry was the best covariance structure based upon the smallest Akaike's information criterion values. Other covariance structures tested included heterogeneous compound symmetry, unstructured, autoregressive 1 and heterogeneous autoregressive 1. Data were subjected to ANOVA, polynomial regression, and means

Table 2. Effects of increasing levels of chitosan on dry matter intake and nutrient digestibility of lactating cows
a,b, Least-squares mean within a row with different letters differ ($P \leq 0.05$)

Item	Treatment[A]				s.e.m.	*P*-value[B]	
	0	75	150	225		LIN	QUA
Dry matter intake (kg/day)	19.8	20.3	19.4	20.2	0.47	0.79	0.57
Dry matter intake (%BW)	3.0	3.0	2.9	3.0	0.08	0.73	0.47
	Coefficient of digestibility (*kg*/*kg*)						
Dry matter	0.650	0.660	0.670	0.670	0.0009	0.29	0.66
Organic matter	0.670	0.680	0.690	0.690	0.0001	0.24	0.56
Crude protein	0.700b	0.720ab	0.731a	0.731a	0.0009	0.05	0.39
Ether extract	0.761	0.801	0.781	0.810	0.0009	0.16	0.66
Neutral detergent fibre	0.601	0.570	0.590	0.580	0.0011	0.47	0.36

[A]Four treatments were evaluated: (1) Control: with no provision of chitosan; (2) 75 mg/kg BW; (3) 150 mg/kg BW; and (4) 225 mg/kg BW of chitosan placed into the rumen through the fistula.
[B]Linear (LIN) or quadratic (QUA) effects.

were separated with LSMEANS, adjusted with Tukey; significance level was set at $P < 0.05$.

Results

Feed intake and nutrient digestibility

Dry matter intake (kg/day and %BW) did not differ ($P \geq 0.47$) among treatments (Table 2). Chitosan provision did not affect ($P \geq 0.16$) DM, organic matter, EE and NDF digestibility. However, chitosan linearly increased ($P = 0.05$) CP digestibility, and the greatest values were observed in cows provided with 150 and 225 mg/kg BW of chitosan.

Ruminal fermentation parameters

Chitosan provision did not affect ($P \geq 0.25$) ruminal pH and NH_3 concentrations (Table 3). There was no chitosan effect ($P \geq 0.25$) on total VFA, acetate, isobutyrate and valerate concentrations. However, chitosan linearly increased ($P = 0.02$) propionate concentrations, and when values were compared by Tukey test, the 225 mg/kg BW treatment had the greatest concentration ($P = 0.05$). Ruminal butyrate concentration linearly decreased ($P = 0.03$) with increased chitosan provision and the greatest value was observed in the 75 mg/kg BW treatment. Isovalerate concentration linearly decreased ($P < 0.01$) with increased chitosan provision. Acetate : propionate ratio linearly reduced ($P = 0.01$) with increased chitosan, and the least value was observed in the 225 mg/kg BW treatment.

Blood metabolites, N balance and microbial protein synthesis

Blood concentrations of glucose, urea, aspartate-succinyltransferase and gamma-glutamyl transferase were not altered ($P \geq 0.30$) by chitosan provision (Table 4). The N intake, excretion in urine and faeces and balance did not differ ($P \geq 0.09$) among treatments. However, chitosan linearly increased ($P = 0.02$) milk N and milk N : N intake ratio, with the greatest values observed in the 225 mg/kg BW treatment. Microbial N, CP and efficiency were not affected ($P \geq 0.44$) by chitosan provision.

Milk yield and composition

Chitosan provision linearly increased ($P = 0.02$) milk, protein and lactose yield with the greatest values observed in the 225 mg/kg BW treatment (Table 5). The fat-corrected milk, fat yield and fat, protein and lactose content in milk were not affected ($P \geq 0.16$). Efficiency of milk production (kg milk/kg DM intake) did not differ ($P \geq 0.18$) among treatments.

Discussion

Despite the fact that chitosan did not affect DM intake, the CP digestibility was improved when cows were provided with chitosan. Araújo *et al.* (2015) reported a linear increase of DM, NDF and CP digestibility without changes in DM intake following provision of increasing doses of chitosan to Nellore steers; however, the authors suggested that the increase of CP digestibility was due to altered fermentation. The mechanism by which chitosan alters CP digestibility is unclear, but can be related to absorption of peptides in the duodenum or the amounts of amino acids escaping ruminal fermentation, once NH_3 ruminal concentration and microbial synthesis were not altered. Some additives can enhance the efficiency of N utilisation by reducing amino acids ruminal deamination rate (Yang and Russell 1993), allowing more amino acids to reach the duodenum for absorption.

There have been several studies examining the effect of chitosan on rumen fermentation, published in the past decade (Goiri *et al.* 2009*a*, 2010; Araújo *et al.* 2015). Goiri *et al.* (2010) reported an increase of *in vitro* propionate and decrease in acetate concentrations when chitosan was used in a substrate of starch but not in cellulose substrate. In the present study, the digestibility of non-fibrous carbohydrate increased (data not shown) for cows provided with chitosan, which could explain the greater propionate production of cows. Araújo *et al.* (2015) also observed a linear increase of propionate concentration when feeding increasing doses of chitosan, and the diet used was similar to the diet of the present study. Furthermore, the

Table 3. Effects of increasing levels of chitosan on ruminal fermentation parameters of lactating cows

a,b, Least-squares mean within a row with different letters differ ($P \leq 0.05$)

Item	Treatment[A]				s.e.m.	*P*-value[B]				
	0	75	150	225		CHI	Time	Int	LIN	QUA
pH	6.42	6.42	6.38	6.34	0.043	0.67	<0.01	0.40	0.25	0.70
NH_3 (mg/dL)	26.70	24.11	25.64	26.93	0.701	0.69	0.77	0.71	0.79	0.30
Total volatile fatty acids (mM)	109.56	103.90	104.53	105.27	2.076	0.65	<0.01	0.65	0.43	0.36
Acetate (mM)	72.29	66.08	68.01	67.42	1.412	0.24	<0.01	0.26	0.20	0.21
Propionate (mM)	19.65b	19.60b	21.56b	23.25a	0.484	0.09	<0.01	0.07	0.02	0.43
Butyrate (mM)	11.69b	13.14a	10.73b	10.33b	0.273	0.02	<0.01	0.72	0.03	0.15
Valerate (mM)	1.52	1.49	1.47	1.55	0.048	0.93	<0.01	0.46	0.86	0.58
Isobutyrate (mM)	1.16a	1.11a	1.02b	1.02b	0.044	0.17	0.22	0.59	0.04	0.64
Isovalerate (mM)	2.52	2.21	1.88	1.80	0.053	<0.01	<0.01	0.26	<0.01	0.19
Acetate : propionate ratio	3.67	3.47	3.25	3.01	0.042	0.01	0.04	0.06	0.01	0.86

[A]Four treatments were evaluated: (1) Control: with no provision of chitosan; (2) 75 mg/kg BW; (3) 150 mg/kg BW; and (4) 225 mg/kg BW of chitosan placed into the rumen through the fistula.

[B]Effects of chitosan (CHI), time, chitosan * time (Int), and linear (LIN) or quadratic (QUA) effects.

Table 4. Effects of increasing levels of chitosan on blood parameters and nitrogen balance of lactating cows
a,b, Least-squares mean within a row with different letters differ ($P \leq 0.05$). AST, aspartate-succinyltransferase (units per litre); GGT, gamma-glutamyltransferase (units per litre)

Item	Treatment[A]				s.e.m.	*P*-value[B]	
	0	75	150	225		LIN	QUA
			Blood metabolites				
Glucose (mg/dL)	83.5	80.7	78.7	77.3	2.62	0.30	0.87
Urea (mg/dL)	30.7	29.9	27.9	29.5	1.25	0.49	0.52
AST (U/L)	58.1	62.6	60.0	60.4	1.75	0.69	0.41
GGT (U/L)	37.4	32.4	37.1	32.3	2.44	0.52	0.99
			Nitrogen balance (*g*/*day*)				
N intake	491.3	503.1	481.3	500.0	11.79	0.92	0.72
Urinary N	153.1	141.7	157.8	155.1	3.98	0.48	0.54
Faecal N	148.7	139.9	128.6	131.8	6.48	0.09	0.45
Milk N	98.7b	100.8b	103.3a	109.7a	4.69	0.02	0.49
N balance	90.9	120.6	91.6	103.4	8.78	0.83	0.31
Milk N: N intake	20.1	20.2	21.4	21.9	0.83	0.02	0.72
Microbial N	224.8	232.6	227.2	238.4	7.13	0.44	0.96
Microbial protein	1405.4	1454.1	1420.1	1490.1	44.58	0.44	0.96
Microbial efficiency[C]	100.1	103.1	102.9	105.3	3.32	0.48	0.92

[A]Four treatments were evaluated: (1) Control: with no provision of chitosan; (2) 75 mg/kg BW; (3) 150 mg/kg BW; and (4) 225 mg/kg BW of chitosan placed into the rumen through the fistula.
[B]Linear (LIN) or quadratic (QUA) effects.
[C]Microbial efficiency = grams of microbial N per kilogram of TDN intake.

Table 5. Effects of increasing levels of chitosan on milk yield and composition of lactating cows
a,b, Least square mean within a row with different letters differ ($P \leq 0.05$)

Item	Treatment[A]				s.e.m.	*P*-value[B]	
	0	75	150	225		LIN	QUA
			Yield (*kg*/*day*)				
Milk	20.06b	20.42b	21.10b	22.20a	0.91	0.02	0.55
3.5% fat-corrected milk	24.40	25.33	25.16	26.24	1.06	0.16	0.93
Fat	0.97	1.01	0.98	1.02	0.04	0.42	0.92
Protein	0.63b	0.64b	0.66ab	0.70a	0.03	0.02	0.46
Lactose	0.95b	0.96b	0.99ab	1.05a	0.04	0.02	0.47
			Composition (%)				
Fat	4.77	5.07	4.65	4.65	0.13	0.16	0.22
Protein	3.13	3.15	3.12	3.14	0.02	0.86	0.69
Lactose	4.71	4.72	4.66	4.70	0.03	0.45	0.45
Milk yield: dry matter intake	1.23	1.27	1.29	1.30	0.05	0.18	0.76
Body condition score[C]	2.78	2.78	2.78	2.80	0.03	0.41	0.48

[A]Four treatments were evaluated: (1) Control: with no provision of chitosan; (2) 75 mg/kg BW; (3) 150 mg/kg BW; and (4) 225 mg/kg BW of chitosan placed into the rumen through the fistula.
[B]Linear (LIN) or quadratic (QUA) effects.
[C]Cow body condition score was estimated using a five-point scale according to Wildman *et al*. (1982), from 1 = emaciated to 5 = obese.

energy efficiency of propionic fermentation is greater when compared with other VFA and can reduce energy diet losses through methane, which is associated with acetic and butyric acids production (Armentano 1992). However, changes in the proportions of VFA produced in the rumen, favouring increased propionate suggests that chitosan is affecting Gram-positive bacteria (McGuffey *et al*. 2001). Generally, chitosan has strong antimicrobial effect against Gram-positive rather than Gram-negative bacteria (Şenel and McClure 2004; Zhong *et al*. 2008). In addition, when the environmental pH is above chitosan pK_a (6.3–6.5), hydrophobic and chelating effects of chitosan are responsible for the antimicrobial activity (Kong *et al*. 2010); however, in the present study, chitosan did not affect ruminal pH.

Chitosan reduced ruminal amino acids deamination. This occurred because of the reduction in ruminal concentration of branched-chain fatty acids as isobutyrate and isovalerate, which are produced in the rumen from deamination of isoleucine, leucine and valine (Chalupa 1980; Horton 1980). This fact could increase amino acids reaching the duodenum and consequently improve N efficiency utilisation. Chitosan increased N excreted in milk without altering N intake and improved N efficiency utilisation. Despite chitosan antimicrobial activity (Kong *et al*. 2010), the microbial protein synthesis was not altered and did not impair milk production in the present study.

Data of productive performance of ruminants provided with chitosan are limited in the literature, but chitosan appears to improve digestion and metabolism, especially by increasing ruminal propionate (Goiri *et al*. 2010; Araújo *et al*. 2015). Milk production, protein and lactose yield of cows were increased when chitosan was provided, mainly because of the greater ruminal propionate production and greater CP digestibility, which led to greater energy and N for milk synthesis.

Conclusion

Chitosan is a natural alternative modulator of ruminal fermentation, which increases ruminal propionate concentration, N utilisation efficiency and milk yield of dairy cows.

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