



## Short communication

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



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## 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 ( $91.3 \pm 15.1$  days in milk,  $29.8 \pm 4.1$  kg/d of milk,  $610 \pm 55$  kg of body weight [BW]) were used in a  $4 \times 4$  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.

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## 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.

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## 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  $91.3 \pm 15.1$  days in milk,  $610 \pm 55$  kg of BW;  $29.8 \pm 4.1$  kg/d of milk yield at the beginning of the trial. Cows were housed in individual pens with  $17.5 \text{ m}^2$ , 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  $4 \times 4$  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<sup>®</sup> 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  $-20^\circ\text{C}$  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  $65^\circ\text{C}$  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) ( $\text{N} \times 6.25$ ; 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 ( $100 \text{ g m}^{-2}$ ) following the recommendation of a maximum of 20 mg of DM/cm<sup>2</sup> ([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  $65^\circ\text{C}$  in a forced-air oven and submitted to acid-detergent solution in an Ankom<sup>®</sup> 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<sup>®</sup>, 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 Electric, 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  $\times$  0.25 mm i.d. with 0.02- $\mu\text{m}$  film thickness; Supelco Sigma-Aldrich Group, Bellefonte, Pennsylvania, USA). The oven temperature was  $70^\circ\text{C}$  for 4 min, increased  $13^\circ\text{C}/\text{min}$  to  $175^\circ\text{C}$ , and then held at this temperature for 27 min. Finally, temperature was increased by  $4^\circ\text{C}/\text{min}$  until reached  $215^\circ\text{C}$ , and kept for 31 min. Hydrogen was used as the carrier gas flowing at  $40 \text{ cm}^3/\text{s}$ . 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 3000g for 10 min. The blood urea nitrogen (BUN) was analyzed with commercially colorimetric kits (urea: cat. no. K-056; Bioclin<sup>®</sup>, 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

to the following statistical model:

$$Y_{iklm} = \mu + C_i + P_k + S_l + A_m(S_l) + e_{iklm}$$

where  $Y_{iklm}$  was the observed value in the animal  $m$ , from  $i$  square, in the  $k$ th period,  $\mu$ =overall mean;  $C_i$  was the fixed effect of chitosan;  $P_k$  was the fixed effect of experimental period;  $S_l$  was the fixed effect of the Latin square;  $A_m(S_l)$  was the random effect of animal within square; and  $e_{iklm}$  was the random residual error. The degrees of freedom were calculated according to Satterthwaite's method (ddfm=satterth). Response to chitosan was tested with linear and quadratic contrasts, and difference were considered significant at  $P \leq 0.05$ .

### 3. Results

There was no effect of CHI on DMI, either in absolute value (kg/d) or relative to BW ( $P > 0.05$ ; Table 1). The addition of CHI linearly increased ( $P=0.01$ ) apparent total-tract digestibility of CP, and quadratically affected ( $P < 0.05$ ) DM, OM and NDF digestibility. Nitrogen intake, N excretion in urine and in milk, and N balance were not affected by treatments ( $P > 0.05$ ). However, N fecal excretion was linearly decreased by CHI ( $P=0.02$ ). Chitosan addition had a quadratic effect ( $P=0.04$ ) on N efficiency (N milk: N intake), and linearly increased ( $P=0.01$ ) blood urea nitrogen. Microbial protein synthesis was similar among treatments ( $P > 0.05$ ).

Increasing doses of CHI did not affect milk yield, fat corrected milk, fat and protein production or content ( $P > 0.05$ ; Table 2). However, milk urea nitrogen (MUN) was linearly increased as CHI addition increased ( $P < 0.05$ ). Body weight and BCS were not affected by CHI levels ( $P > 0.05$ ). Chitosan linearly decreased ( $P < 0.05$ ) C6 and C18:1 c9 FA, and quadratically affected C14:1 FA concentration in milk fat (Table 3). Nevertheless, there was no effect of CHI on < C16, C16, > C16 total FA concentrations in milk fat ( $P > 0.05$ ). Total of C18 saturated, unsaturated and unsaturated to saturated FA ratio were similar among treatments ( $P > 0.05$ ).

**Table 1**

Dry matter intake, apparent total-tract digestibility and nitrogen balance in dairy cows supplemented with chitosan.

Item	Treatments <sup>a</sup>				SEM <sup>b</sup>	P-value <sup>c</sup>	
	C0	C50	C100	C150		LIN	QUA
Dry matter intake, kg/d	24.49	24.82	24.26	24.07	0.44	0.34	0.55
Dry matter intake, % BW	3.96	4.01	3.92	3.89	0.07	0.37	0.56
<i>Total tract apparent digestibility (%)</i>							
Dry matter	67.22	70.91	69.51	69.35	0.70	0.28	0.05
Organic matter	67.99	71.88	70.61	70.06	0.81	0.25	0.03
Crude protein	78.79	80.92	81.29	81.53	0.57	0.01	0.18
Neutral detergent fiber	56.44	62.13	60.28	58.84	0.93	0.36	0.01
<i>Nitrogen balance</i>							
N intake, g/d	645.33	655.07	639.83	638.45	10.8	0.47	0.61
N urinary, g/d	283.73	300.28	289.03	290.40	5.48	0.76	0.24
N fecal, g/d	137.36	126.37	120.39	120.48	4.52	0.02	0.31
N milk, g/d	142.23	141.20	141.59	147.44	2.21	0.24	0.26
N balance, g/d	82.14	87.55	88.55	80.00	84.5	0.82	0.21
N milk: N intake	22.3	21.7	22.4	23.4	0.39	0.03	0.04
BUN <sup>d</sup> , mg/dL	38.59	40.16	41.94	44.35	1.05	0.01	0.79
Microbial protein, g/d	1818.6	1860.1	1776.6	1766.2	36.2	0.33	0.64

<sup>a</sup> C0=control treatment, without chitosan (CHI) addition; C50, C100 and C150=supplemented of 50, 100 and 150 mg/kg of body weight (BW) chitosan, respectively.

<sup>b</sup> SEM=standard error of the mean.

<sup>c</sup> LIN and QUA=probability for linear and quadratic effect, respectively.

<sup>d</sup> Blood urea nitrogen.

**Table 2**

Milk yield and composition of dairy cows supplemented with increasing doses of chitosan.

Item	Treatments <sup>a</sup>				SEM <sup>b</sup>	P-value <sup>c</sup>	
	C0	C50	C100	C150		LIN	QUA
Milk yield, kg/d	29.71	29.38	29.46	29.85	0.51	0.73	0.27
3.5% FCM <sup>d</sup> , kg/d	26.11	26.23	27.00	27.31	0.55	0.12	0.88
Fat, kg/d	0.82	0.83	0.87	0.89	0.02	0.16	0.91
Protein, kg/d	0.90	0.89	0.90	0.94	0.01	0.28	0.25
Fat, %	2.75	2.86	2.98	2.97	0.08	0.19	0.65
Protein, %	3.07	3.08	3.06	3.16	0.02	0.25	0.35
MUN <sup>e</sup> , mg/dL	8.20	8.59	8.63	10.33	0.25	< 0.01	0.15
Body weight, kg	616	632	604	614	7.26	0.29	0.70
BCS <sup>f</sup>	2.7	2.7	2.7	2.7	0.05	0.69	0.24

<sup>a</sup> C0=control treatment, without chitosan (CHI) addition; C50, C100 and C150=supplemented with 50, 100 and 150 mg/kg of body weight (BW) of chitosan, respectively.

<sup>b</sup> SEM=standard error of the mean.

<sup>c</sup> LIN and QUA=probability for linear and quadratic effects, respectively.

<sup>d</sup> FCM=fat corrected milk.

<sup>e</sup> Milk urea nitrogen.

<sup>f</sup> Body condition score (1–5).

**Table 3**

Milk fatty acids profile of dairy cows supplemented with increasing doses of chitosan.

Item	Treatments <sup>a</sup>				SEM <sup>b</sup>	P-value <sup>c</sup>	
	C0	C50	C100	C150		LIN	QUA
Fatty acid	<i>g/100 g total fatty acids</i>						
4:0	0.93	0.76	0.99	0.66	0.07	0.33	0.55
6:0	1.55	1.40	1.50	1.18	0.04	0.01	0.33
8:0	1.18	1.13	1.12	1.09	0.02	0.12	0.73
10:0	2.91	2.89	2.77	2.89	0.06	0.71	0.58
11:0	0.29	0.27	0.21	0.24	0.01	0.11	0.34
12:0	3.40	3.34	3.19	3.40	0.07	0.84	0.39
13:0	0.11	0.13	0.11	0.12	0.01	0.83	0.64
14:0	11.13	10.91	10.64	11.43	0.13	0.59	0.06
14:1	0.54	0.51	0.53	0.58	0.01	0.06	0.02
15:0	0.14	0.16	0.15	0.15	0.01	0.45	0.59
16:0	29.62	30.16	29.39	31.90	0.49	0.07	0.18
16:1 cis	0.97	0.98	0.88	0.95	0.02	0.19	0.41
17:0	0.25	0.27	0.26	0.27	0.01	0.22	0.73
17:1	0.10	0.08	0.08	0.09	0.01	0.74	0.49
18:0	13.50	13.97	14.72	13.47	0.33	0.79	0.13
18:1 trans-11	0.39	0.41	0.47	0.44	0.01	0.11	0.37
18:1 cis-9	24.04	23.28	23.51	21.68	0.41	0.04	0.46
18:2 n-6, cis	2.96	3.10	2.98	3.05	0.06	0.68	0.63
18:3	0.13	0.14	0.14	0.14	0.01	0.29	0.95
20:0	0.11	0.10	0.12	0.10	0.01	0.60	0.41
20:1	0.20	0.20	0.19	0.19	0.01	0.22	0.79
Other	5.27	6.25	5.68	5.55	0.13	0.80	0.02
Total							
< C16	22.21	21.55	21.59	21.45	0.37	0.50	0.72
C16	30.61	31.17	30.27	32.90	0.48	0.08	0.17
> C16	41.80	41.50	42.37	40.19	0.73	0.48	0.44
<i>C18</i>							
Unsaturated C18	27.52	26.90	27.08	25.28	0.45	0.06	0.45
Saturated C18	13.50	13.97	14.72	13.47	0.33	0.79	0.13
Unsaturated: saturated C18	2.06	1.96	1.89	2.00	0.04	0.48	0.21
Total							
Unsaturated	29.28	28.67	28.79	27.04	0.46	0.06	0.46
Saturated	65.15	65.55	65.66	66.61	0.52	0.30	0.72
Unsaturated: saturated	0.45	0.44	0.44	0.40	0.01	0.09	0.46

<sup>a</sup> C0=control treatment, without chitosan (CHI) addition; C50, C100 and C150=supplemented with 50, 100 and 150 mg/kg of body weight (BW) of chitosan, respectively.

<sup>b</sup> SEM=standard error of the mean.

<sup>c</sup> LIN and QUA=probability for linear and quadratic effects, respectively.

Furthermore, CHI had no effect ( $P > 0.05$ ) on total saturated and unsaturated FA concentration in milk.

#### 4. Discussion

This study was aimed to evaluate the effects of CHI on feed intake, nutrient digestibility and performance in lactating dairy cows. In the current study, changes in digestibility of DM and NDF occurred, but they were not sufficient to alter DMI. Previous studies with increasing doses of CHI reported similar results, with improved nutrient digestibility without affect DMI (Araújo et al., 2015; Paiva et al., 2016) and the effects are probably related to altered ruminal fermentation.

Apparent total-tract digestibility of DM, CP and NDF improved when CHI was added to diet, in the current experiment. Similar results were observed when CHI was supplied to beef steers (Araújo et al., 2015). However, *in vitro* studies reported negative effects of CHI including decreased DM and NDF digestibility, especially in high-fiber mixtures in batch cultures (Goiri et al., 2009a, 2009b). Moreover, in an *in vivo* study with sheep, CHI tended to decrease NDF digestibility but without affect OM digestibility (Goiri et al., 2010a). The CHI mechanism of action is still not clear, but ionic interactions between positively charged bacterial surface chitosan amino group and negatively charged in the surface of bacterial, resulting in alteration of membrane permeability, has been the most acceptable theory (Helander et al., 2001; Kong et al., 2010). Zhong et al. (2008) reported that Gram-positive bacteria are more susceptible to derivatives of CHI as a consequence of the Gram-negative outer membrane barrier. The increase in protein digestibility could be related to the action of CHI on bacteria which promote proteolysis and deamination, resulting in a decrease of ruminal protein degradation and increasing amino acids availability in the intestine, a similar effect of ionophores (Russell and Martin, 1984; Yang and Russell, 1993). Thus, we suggest that the increase of the nutrient digestibility with CHI addition its due its capacity to change rumen microorganisms and digestive processes, acting mainly on the Gram-positive bacteria, that is justified by improvement in NDF and CP digestibility.

In the current study, CHI had increased BUN and milk urea nitrogen, and reduced N fecal excretion without affecting N balance and microbial protein synthesis. This fact can be related to the increase in protein digestibility, associated with the hepatic metabolism of nitrogen compounds together with urinary and fecal excretion (Jonker et al., 1998; Spek et al., 2013), leading to urea accumulation in the serum of these animals and consequently excreted in the milk. Moreover, Garcia-Rodriguez et al. (2011) reported increased BUN of sheep supplemented with CHI, which may be related to higher degradability of protein in the rumen. In this study, the reduction of N fecal excretion with CHI supplementation is likely due to the better utilization of nitrogen in amino acids available in the small intestine resulting from changes in rumen fermentation caused by CHI, which is related to improvement in protein digestibility. Therefore, when protein from feed is more digestible than those from microbial origin, amino acid uptake by the small intestine may be increased (McGuffey et al., 2001; Ruiz et al., 2001).

Milk production and composition were not affected by CHI, which could be related to DMI that was not affected in this study; thus, these findings indicate that supply of nutrients to cows and especially to mammary gland was not limited. Paiva et al. (2016) reported that CHI increased milk yield and this effect was related to improvements in nutrient digestibility and ruminal fermentation, especially by increasing ruminal propionate which led to greater energy available for milk production.

Few milk FA were affected by CHI (C6:0; C14:1 and C18:1 c9) in

the current study. However, in an *in vitro* study CHI decreased total C18:0 and increased total C18:1, C18:1 t11, total CLA, and the C18 unsaturated to saturated FA ratio (Goiri et al., 2010b). Milk FA are derived almost equally from two sources, the feed and the microbial activity on the rumen (Parodi, 2004). According to Goiri et al. (2010b), CHI could be interacting with negatively charged free FA, thereby preventing the biohydrogenation process, and/or could be affecting certain microbial population's growth. Another reason which may explain the absence of CHI effects on FA biohydrogenation in the current study is the level of EE in the diet (4.23%) and major FA source (whole raw soybean) used. Goiri et al. (2010b) used rapeseed and sunflower meals as FA sources, which can release FA in the rumen more easily compared to whole raw soybean. Indeed, in high fat diets the content of free FA is quickly biohydrogenated in the rumen due to faster action of lipases, turning free FA available to hydrogen addition that leads to formation of CLA isomers (Doreau, 2011). Thus, our results suggest that CHI promote changes in the ruminal fermentation without alter milk FA profile when oilseeds was used as the dietary FA source.

#### 5. Conclusion

Chitosan improved nutrient digestibility without altering productive performance and milk fatty acid profile in mid-lactation dairy cows. The results indicate that better doses of chitosan inclusion are between 100 and 150 mg/kg BW.

#### Conflict of interest statement

None authors of this manuscript has declared any conflict of interest statement.

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#### Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.livsci.2016.02.008>.

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