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Nutrient digestion, microbial protein synthesis, and blood metabolites of Jersey heifers fed chitosan and whole raw soybeans

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ABSTRACT - This study was undertaken to determine the effects of chitosan and whole raw soybean on nutrient intake and total tract digestion, nitrogen utilization, microbial protein synthesis, blood metabolites, and energy balance of dairy heifers. Twelve Jersey heifers (6±0.5 months of age and 139.50±25.56 kg of live weight; mean ± standard deviation) were randomly assigned to a replicated Latin square design with a 2 × 2 factorial arrangement. The experimental period consisted of 14 days of adaptation to diets, six days of sampling, and five days of washout. The experimental diets were: control (CO); chitosan (CHI, inclusion of 2.0 g kg^{-1} DM of chitosan); whole raw soybean (WS, 163.0 g kg^{-1} of WS on diet DM basis); and chitosan + whole raw soybean (CHI+WS). Chitosan decreased dry matter and neutral detergent fiber intakes; however, CHI increased DM total tract digestion. An interaction effect was observed on retained nitrogen, which increased when animals were fed CHI+WS compared with CO or CHI, but did not differ from that of animals fed WS. Chitosan decreased microbial nitrogen and crude protein flow of heifers. Energy balance was improved when heifers received diets containing WS. Efficiency of energy utilization was not affected by experimental diets. An interaction effect was observed for blood high-density lipoprotein (HDL) concentration, which increased with both dietary inclusion of CHI and WS compared with the other diets, and CHI provided the lowest value of HDL cholesterol. Chitosan and whole raw soybean do not alter nutrient intake and total tract digestion; however, they decrease nitrogen urinary excretion and increase blood HDL cholesterol of heifers.

Key Words: antimicrobial, nitrogen metabolism, oilseed, rumen modulator

Introduction

The rising feed costs and the necessity to improve the feed conversion ratio have increased the number of studies aimed at limiting the feed intake and increasing the dietary nutrient density (Hoffman et al., 2007). Whole raw soybean (WS) is commonly used as a source of supplementary fat and protein and is considered an economical and convenient source of nutrients (NRC, 2001). Furthermore, the lipid fraction contained in the WS is slowly released in the rumen environment due to the protein complex that protects the oil contained in the cotyledon of seeds, and consequently may not impair ruminal fiber digestion. In addition to the soybean availability, feeding WS decreases costs with taxes and fees and losses during the industrial process, transportation, and storage. To our knowledge, no studies with WS inclusion in the diet of dairy heifers are reported in literature. However, Venturelli et al. (2015) found that increasing dietary levels of WS decreased dry matter (DM) intake and maintained 3.5% fat-corrected milk yield of Holstein cows.

Another way to improve the performance of heifers is by using feed additives with antimicrobial activity to shift ruminal fermentation to a more energetically efficient pathway. Goiri et al. (2009) proposed the utilization of chitosan (CHI) to modulate ruminal fermentation and digestion with promising results. Chitosan is a natural biopolymer derived from the deacetylation of chitin (Goiri et al., 2009). The antimicrobial activity of CHI is well known against bacteria and fungi (Senel and McClure, 2004). However, the utilization of CHI in animal feeding has been underexploited, and there are few studies available in literature. Araújo et al. (2015) reported a linear increase in the digestibility of DM, crude protein (CP), and neutral detergent fiber (NDF) when beef steers were fed CHI, without changing their DM intake.

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The objective of the present experiment was to determine the effects of dietary inclusion of WS and CHI on nutrient intake and total tract digestion, nitrogen utilization, microbial protein synthesis, and blood metabolites of dairy heifers. Our hypothesis was that feeding both WS and CHI would improve nutrient total tract digestion and utilization by dairy heifers.

Material and Methods

This study was approved by the Bioethics Committee of Universidade Federal da Grande Dourados, located in Dourados - MS, Brazil. Twelve Jersey heifers (6±0.5 months of age and 139.50±25.56 kg of live weight; mean ± standard deviation) were randomly assigned to a balanced (according to the body weight) and contemporary replicated Latin square design, with a 2 × 2 factorial dietary arrangement. The experimental periods consisted of 14 days of adaptation to diets, six days of sampling, and five days of washout. Animals were allocated in individual pens of 8 m^2 throughout the experiment.

The following experimental diets were used: control (CO); chitosan (CHI, inclusion of 2.0 g kg^{-1} DM of chitosan); whole raw soybean (WS, 163.0 g kg^{-1} DM of WS); and chitosan + whole raw soybean (CHI+WS). The diets, formulated to provide an average daily gain of 700.0 g d^{-1} according to NRC (2001), were isonitrogenous and contained corn silage as the forage source (Table 1). Chitosan presented the following technical specifications: apparent density of 0.64 g mL^{-1}, 20 g kg^{-1} of ash, 7.0-9.0 of pH, viscosity <200 cPs, and deacetylation level of 95% (Polymar Industria e Cia. Imp. and Exp. Ltda., Ceara, Brazil). Diets were fed as a total mixed ration twice daily at 06.30 h and at 13.00 h. Amounts of feed offered and orts for each heifer were weighed daily and orts were restricted to 5 to 10% of intake on an as-fed basis.

Samples of all diet ingredients (0.5 kg) and orts (125.0 g kg^{-1} of total daily orts) from each heifer were collected during the last six days of each period and combined into one composite sample of orts for each cow and one composite sample of silage. Samples were analyzed to determine dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber, lignin, and ash according to AOAC (2000). Total feces collection was performed for a 24-h period on days 15, 16, and 17 of each experimental period from each heifer, and then feces were homogenized and aliquots of 10% (wet basis) were frozen at −20 ºC until analyses.

Urine samples were collected from each heifer 4 h after feeding on day 14 of each experimental period. The urine was filtered and 10 mL aliquots were immediately diluted in 40 mL of sulfuric acid (0.036 N) to prevent the bacterial destruction of purine derivatives and uric acid precipitation. A 50 mL urine sample with 1 mL of sulfuric acid (0.036 N) was stored for nitrogen, urea, and creatinine determination. Creatinine concentrations were determined by the enzymatic colorimetric method using commercial kits (Laborlab®, Osasco, Brazil) and reading was performed in an automatic biochemistry analyzer (SBA-200 automatic biochemistry, CELM®, Sao Caetano do Sul, Brazil). The allantoin and uric acid concentrations in urine were determined by the colorimetric method according to the methodology of Fujihara et al. (1987), described by Chen and Gomes (1992). Total daily urinary volume was estimated as the ratio between creatinine excretion and creatinine concentration contained in the spot urine sample, according to Oliveira et al. (2001).

Samples of ingredients were analyzed in a bomb calorimeter to obtain the gross energy intake and calculate the energy efficiency, according to Harvatine and Allen (2006). Digestible energy intake was obtained based on the digestibility coefficient of experimental diets and gross energy intake, according to the energy values obtained for

Table 1 - Ingredients and chemical composition of the experimental diets

Item	Diet[1]			
	CO	CHI	WS	CHI+WS
Ingredient (g kg^{-1} DM)				
Corn silage	500.4	500.4	500.4	500.4
Ground corn	248.4	248.4	195.0	195.0
Soybean meal	200.1	200.1	90.5	90.5
Whole raw soybean	-	-	163.0	163.0
Mineral mixture[2]	51.1	51.1	51.1	51.1
Chitosan	-	2.0	-	2.0
Chemical composition (g kg^{-1} DM)				
Dry matter, as-fed	573.0	573.0	575.5	575.5
Organic matter	950.3	950.3	948.2	948.2
Crude protein	149.5	149.5	149.0	149.0
Ether extract	24.8	24.8	72.0	72.0
Neutral detergent fiber	378.3	378.3	383.8	383.8
Non-fiber carbohydrates[3]	397.7	397.7	336.9	336.9
Ash	49.3	49.3	51.4	51.4
Total digestible nutrients[4]	710.0	710.0	774.3	774.3
Net energy[4]	1.62	1.62	1.78	1.78
Net energy for gain[4]	1.20	1.20	1.39	1.39

DM - dry matter; EE - ether extract; NFC - non-fiber carbohydrates; CP - crude protein; NDF - neutral detergent fiber.
[1] CO - control; CHI - chitosan, addition of 2 g kg^{-1} diet DM of chitosan; WS - whole raw soybeans, diet containing 72 g kg^{-1} EE on diet DM basis; CHI+WS - chitosan and whole raw soybeans, addition of 2 g kg^{-1} diet DM of chitosan and diet containing 72 g kg^{-1} EE.
[2] Contains per kilogram: 120.00 g Ca; 88.00 g P; 75.00 mg I; 1,300.00 mg Mn; 126.00 g Na; 15.00 mg Se; 12.00 mg S; 3,630.00 mg Co; 55.50 mg Cu; and 1,800.00 mg Fe.
[3] NFC = 100 − [(%CP − %CP from urea + %urea) + %EE +%ash +%NDF], according to Hall (1998).
[4] Calculated according to the NRC (2001) model.

the ingredients (Harvatine and Allen, 2006). The values of net energy intake, net energy for gain, and net energy for maintenance were calculated according to NRC (2001). At the start of experiment and on day 15 of each period, animals were weighed on a livestock scale for large animals.

Total excretion of purine derivatives was calculated as the sum of allantoin and uric acid excreted in urine, expressed in mmol day^{-1}. The absorbed microbial purines (Pabs, mmol d^{-1}) were calculated from the excretion of purine derivatives (PD, mmol/day) based on the following equation: $Pabs = (PD - 0.512*LW^{0.75})/0.70$, in which 0.70 is the recovery of absorbed purines as purine derivatives and $0.512*LW^{0.75}$ is the endogenous excretion of purine derivatives (González-Ronquillo et al., 2003). Ruminal synthesis of nitrogenous compounds (Nmic, g N d^{-1}) was calculated based on absorbed purines (Pabs, mmol d^{-1}), using the following equation (Chen and Gomes, 1992): $Nmic = (70*Pabs)/(0.83*0.134*1000)$, in which 70 is the nitrogen content in purines (mg N mol^{-1}); 0.134 is the N from purine:total bacterial N ratio (Valadares et al., 1999); and 0.83 is the intestinal digestibility of microbial purines. Nitrogen balance was estimated by subtracting fecal and urinary nitrogen values from total nitrogen intake.

Blood samples were collected from all heifers in sterile Vacutainer® tubes by puncture of the coccygeal vein on day 14 of each experimental period, before the morning feeding. Blood samples were immediately centrifuged for 15 min at 2000 × *g*, and the supernatant was transferred to labeled plastic tubes and stored at −20 ºC. Creatinine and urea concentrations in the blood were determined by the colorimetric method using commercial kits (Laborlab®, Osasco, Brazil). The plasma urea nitrogen concentration was obtained as the urea blood concentration multiplied by 0.466 (N content of urea). Plasma creatinine nitrogen concentration was obtained by multiplying the concentration of creatinine in the plasma by 0.3715 (N content of creatinine). The plasma depuration or clearance of creatinine and urea was obtained as the ratio between the urinary excretion for 24 h and the plasma concentration of each substance. The excreted fraction of urea was determined as the ratio between the depurations of plasma urea and creatinine.

Data were subjected to analysis of variance using the PROC MIXED procedure of SAS (Statistical Analysis System, version 9.1.3), checking the normality of residuals and homogeneity of variances using PROC UNIVARIATE procedure, according to the following model:

$$Y_{ijkl} = \mu + a_i + P_j + C_k + W_l + C_kW_l + P_jC_k + P_jW_k + e_{ijkl},$$

in which: Y_{ijkl} = dependent variable; μ = overall mean; a_i = animal effect; P_j = fixed effect of period; C_k = fixed effect of chitosan; W_l = fixed effect of whole raw soybean; C_kW_l = chitosan*whole raw soybean interaction fixed effect; P_jC_k = period*chitosan interaction fixed effect; P_jW_k = period*whole raw soybean fixed effect; and e_{ijkl} = residual error. The degrees of freedom were calculated as DDFM = kr. Significance level was set at 0.05. The PDIFF test was applied when an interaction effect was observed to determine differences among treatments.

Results

As expected, control and CHI diets showed a higher non-fiber carbohydrate (NFC) content and lower total digestible nutrients (TDN) compared with diets containing WS. Ether extract content in fat-supplemented diets was 72 g kg^{-1} (Table 1).

Chitosan decreased (P≤0.022) DM and NDF intake (Table 2). In addition, CHI increased (P = 0.001) DM total tract digestion. Whole raw soybean decreased (P = 0.001) NFC intake and increased ether extract intake (P = 0.001). Moreover, WS increased EE total tract digestion (P = 0.012). No interaction effects were observed on nutrient intake and total tract digestion. Chitosan decreased (P = 0.005) fecal nitrogen excretion (Table 3). An interaction effect (P = 0.004) was observed on nitrogen excretion in urine, which was lower when heifers were fed chitosan associated with supplemental fat compared with CO or CHI, but did not differ from animals fed WS. Furthermore, an interaction effect was observed on retained nitrogen, which increased when animals were fed CHI+WS compared with those fed CO or CHI, but did not differ from that of animals fed WS.

Gross energy, metabolizable energy, and net energy intake were higher (P≤0.033) in heifers fed WS compared with the other experimental diets. Energy balance was improved when heifers received diet containing WS (P = 0.002). Efficiency of energy utilization was not affected by experimental diets.

Chitosan decreased (P≤0.023) total purine daily production, absorbable purines, microbial nitrogen, and crude protein flow of heifers (Table 4). Supplemental fat did not alter microbial protein synthesis of dairy heifers. An interaction effect (P = 0.024) was observed on uric acid, which increased when heifers were fed CHI+WS in relation to those fed CO or WS; animals fed CHI presented the lowest value of uric acid.

No interaction effects were observed on nitrogen compounds of heifers. However, CHI decreased (P = 0.023) blood urea and urea nitrogen concentrations, and increased (P = 0.008) blood creatinine and creatinine nitrogen concentrations (Table 5). Chitosan also decreased (P = 0.009) creatinine clearance and increased (P = 0.003) the

fractional excretion of urea. Whole raw soybean increased (P = 0.001) blood urea and decreased (P = 0.012) creatinine concentrations. Consequently, WS decreased (P = 0.001) urea clearance and increased (P = 0.027) creatinine clearance.

Chitosan decreased (P≤0.002) total and low-density lipoprotein (LDL) cholesterol (Table 6), contrary to WS, which increased (P≤0.004) total and LDL cholesterol concentrations in blood. An interaction effect (P = 0.006) was observed for blood high-density lipoprotein (HDL) concentration, which increased with both dietary inclusion of chitosan and WS compared with the other diets. Animals fed chitosan showed the lowest value of HDL cholesterol.

Table 2 - Nutrient intake and total tract digestion of Jersey heifers fed chitosan and whole raw soybeans

Item	Diet[1]				SEM	P-value[2]		
	CO	CHI	WS	CHI+WS		CHI	WS	INT
Intake (kg d^{-1})								
Dry matter	6.45	5.66	5.97	5.86	0.20	0.022	0.186	0.198
Organic matter	6.14	5.38	5.67	5.56	0.19	0.043	0.301	0.112
Crude protein	1.06	1.00	0.99	1.00	0.03	0.678	0.277	0.118
Neutral detergent fiber	2.21	1.90	2.05	1.92	0.07	0.009	0.222	0.177
Non-fiber carbohydrate	2.75	2.57	2.16	2.18	0.09	0.167	0.001	0.225
Ether extract	0.172	0.163	0.513	0.523	0.03	0.449	0.001	0.449
Intake (kg/100 kg of LW)								
Dry matter	4.50	4.07	3.55	3.45	0.11	0.100	0.001	0.208
Neutral detergent fiber	1.92	1.78	1.29	1.28	0.06	0.178	0.001	0.308
Total tract digestion (g kg^{-1})								
Dry matter	677.3	692.0	580.1	583.4	1.59	0.001	0.572	0.770
Organic matter	698.7	709.9	615.9	618.6	1.45	0.006	0.665	0.751
Crude protein	755.0	770.8	753.4	758.4	1.08	0.443	0.661	0.548
Neutral detergent fiber	584.9	599.1	555.7	547.9	2.42	0.560	0.312	0.654
Ether extract	891.8	879.9	926.3	886.7	0.41	0.342	0.012	0.456

SEM - standard error of the mean; LW - live weight; DM - dry matter; EE - ether extract.
[1] CO - control; CHI - chitosan, addition of 2 g kg^{-1} diet DM of chitosan; WS - whole raw soybeans, diet containing 72 g kg^{-1} EE on diet DM basis; CHI+WS - chitosan and whole raw soybeans, addition of 2 g kg^{-1} diet DM of chitosan and diet containing 72 g kg^{-1} EE.
[2] Effects of chitosan (CHI), whole raw soybeans (WS), and interaction between CHI and WS (INT).

Table 3 - Efficiency of nitrogen and energy utilization by Jersey heifers fed chitosan and whole raw soybeans

Item	Diet[1]				SEM	P-value[2]		
	CO	CHI	WS	CHI+WS		CHI	WS	INT
Nitrogen balance (g d^{-1})								
N intake	169.49	160.64	158.15	160.27	5.56	0.341	0.216	0.188
N feces	40.02	36.62	38.69	37.87	1.66	0.005	0.546	0.504
N urine	100.75a	99.21a	33.69b	30.35b	13.59	0.022	0.001	0.004
N absorbed	129.47	124.02	119.45	122.40	5.21	0.148	0.342	0.323
N retained	28.71b	24.80b	85.76a	92.03a	15.24	0.001	0.016	0.005
Intake (Mcal d^{-1})								
Gross energy	26.95	25.95	29.79	29.47	0.98	0.096	0.008	0.182
Digestible energy	20.91	19.25	21.28	21.05	0.70	0.087	0.091	0.189
Metabolizable energy	18.25	16.84	18.84	18.68	0.62	0.200	0.033	0.289
Net energy	8.37	7.83	9.06	9.08	0.30	0.252	0.003	0.186
Production (Mcal d^{-1})								
Maintenance	3.56	3.51	3.56	3.57	0.11	0.547	0.155	0.189
Growth	2.25	2.25	2.26	2.26	0.07	0.814	0.871	0.957
Balance (Mcal d^{-1})	2.55	2.06	3.23	3.26	0.19	0.217	0.002	0.135
Efficiency of energy utilization (%)								
NE_g/DE	10.93	11.73	10.63	10.76	0.25	0.139	0.115	0.263
NE_m+NE_g/DE	28.25	30.10	27.48	27.83	0.65	0.189	0.150	0.377

SEM - standard error of the mean; DM - dry matter; EE - ether extract.
NE_g - net energy for gain; DE - digestible energy; NE_m - met energy for maintenance.
[1] CO - control; CHI - chitosan, addition of 2 g kg^{-1} diet DM of chitosan; WS - whole raw soybeans, diet containing 72 g kg^{-1} EE on diet DM basis; CHI+WS - chitosan and whole raw soybeans, addition of 2 g kg^{-1} diet DM of chitosan and diet containing 72 g kg^{-1} EE.
[2] Effects of chitosan (CHI), whole raw soybeans (WS), and interaction between CHI and WS (INT).
a-c - values in the same row with a different letter differ significantly at P≤0.05 according to the PDIFF test.

Table 4 - Microbial protein synthesis of Jersey heifers fed chitosan and whole raw soybeans

Item	Diet[1]				SEM	P-value[2]		
	CO	CHI	WS	CHI+WS		CHI	WS	INT
Allantoin (mmol L^{-1})	5.81	5.01	4.67	4.58	0.43	0.440	0.165	0.426
Uric acid (mmol L^{-1})	2.82a	1.74c	2.23b	3.13a	0.27	0.553	0.336	0.024
Total purines (mmol L^{-1})	8.64	6.76	6.90	7.71	0.49	0.547	0.441	0.059
Allantoin (mmol d^{-1})	84.09	64.23	84.78	59.19	7.56	0.055	0.668	0.775
Uric acid (mmol d^{-1})	38.24	20.28	44.71	39.88	4.28	0.096	0.113	0.229
Total purines (mmol d^{-1})	122.33	84.51	129.48	99.07	8.92	0.013	0.378	0.773
Absorbable purines (mmol d^{-1})	137.70	93.10	145.97	110.28	10.56	0.009	0.235	0.554
Microbial flow								
Nitrogen (g d^{-1})	103.86	71.09	110.00	83.71	7.74	0.023	0.445	0.678
Crude protein (g d^{-1})	649.12	444.33	687.52	523.18	48.38	0.023	0.445	0.678

SEM - standard error of the mean; DM - dry matter; EE - ether extract.
[1] CO - control; CHI - chitosan, addition of 2 g kg^{-1} diet DM of chitosan; WS - whole raw soybeans, diet containing 72 g kg^{-1} EE on diet DM basis; CHI+WS - chitosan and whole raw soybeans, addition of 2 g kg^{-1} diet DM of chitosan and diet containing 72 g kg^{-1} EE.
[2] Effects of chitosan (CHI), whole raw soybeans (WS), and interaction between CHI and WS (INT).
a-c - values in the same row with a different letter differ significantly at P≤0.05 according to the PDIFF test.

Table 5 - Nitrogen compounds of Jersey heifers fed chitosan and whole raw soybeans

Item	Diet[1]				SEM	P-value[2]		
	CO	CHI	WS	CHI+WS		CHI	WS	INT
Urine (mg dL^{-1})								
Urea	125.63	125.63	121.38	118.38	2.59	0.553	0.060	0.453
Creatinine	4.23	3.98	3.35	5.00	0.36	0.167	0.845	0.100
Urea nitrogen	58.54	58.54	56.56	55.16	1.21	0.553	0.060	0.453
Creatinine nitrogen	1.57	1.48	1.24	1.85	0.13	0.167	0.845	0.100
Blood (mg dL^{-1})								
Urea	40.37	35.50	43.12	42.37	2.30	0.023	0.001	0.100
Creatinine	0.77	0.80	0.53	0.78	0.04	0.008	0.012	0.182
Urea nitrogen	18.81	16.54	20.09	19.74	1.07	0.023	0.001	0.100
Creatinine nitrogen	0.28	0.29	0.20	0.29	0.01	0.008	0.012	0.182
Excretion (mg kg of LW^{-1})								
Urea	506.15	485.90	451.46	443.31	27.11	0.602	0.057	0.662
Creatinine	30.52	30.56	30.52	30.52	0.07	0.192	0.207	0.112
Clearance 24 h (%)								
Urea	14.18	15.32	11.35	11.16	1.11	0.504	0.001	0.540
Creatinine	44.40	41.68	60.19	42.03	2.76	0.009	0.027	0.049
Fractional excretion (%)								
Urea	30.91	36.18	20.09	26.78	1.96	0.003	0.001	0.652

SEM - standard error of the mean; LW - live weight; DM - dry matter; EE - ether extract.
[1] CO - control; CHI - chitosan, addition of 2 g kg^{-1} diet DM of chitosan; WS - whole raw soybeans, diet containing 72 g kg^{-1} EE on diet DM basis; CHI+WS - chitosan and whole raw soybeans, addition of 2 g kg^{-1} diet DM of chitosan and diet containing 72 g kg^{-1} EE.
[2] Effects of chitosan (CHI), whole raw soybeans (WS), and interaction between CHI and WS (INT).

Table 6 - Blood metabolites of Jersey heifers fed chitosan and whole raw soybeans

Item	Diet[1]				SEM	P-value[2]		
	CO	CHI	WS	CHI+WS		CHI	WS	INT
Glucose (mg dL^{-1})	98.68	96.01	93.15	99.32	5.39	0.771	0.657	0.667
Triacylglycerol (mg dL^{-1})	50.25	51.12	30.12	49.12	7.83	0.547	0.349	0.407
Total cholesterol (mg dL^{-1})	91.25	78.12	131.25	110.12	13.51	0.001	0.001	0.334
HDL	28.00b	23.75c	32.87b	46.37a	3.60	0.367	0.099	0.006
LDL	53.20	44.15	92.35	53.92	11.19	0.002	0.004	0.426
VLDL	10.05	10.22	6.02	9.82	1.56	0.227	0.329	0.327

SEM - standard error of the mean; DM - dry matter; EE - ether extract.
HDL - high-density lipoprotein; LDL - low-density lipoprotein; VLDL - very-low density lipoprotein.
[1] CO - control; CHI - chitosan, addition of 2 g kg^{-1} diet DM of chitosan; WS - whole raw soybeans, diet containing 72 g kg^{-1} EE on diet DM basis; CHI+WS - chitosan and whole raw soybeans, addition of 2 g kg^{-1} diet DM of chitosan and diet containing 72 g kg^{-1} EE.
[2] Effects of chitosan (CHI), whole raw soybeans (WS), and interaction between CHI and WS (INT).
a-c - values in the same row with a different letter differ significantly at P≤0.05 according to the PDIFF test.

Discussion

Chitosan decreased the intakes of DM and NDF and increased DM total tract digestion (Table 2). Dry matter intake is a function of meal size and meal frequency, which are determined by dietary and animal factors that alter hunger and satiety (Allen, 2000). Decreased DM intake changes the ruminal nutrient passage, and the feed would be available for longer periods in the ruminal environment, increasing ruminal nutrient digestion. There is evidence that oxidizable fuels in the liver affect feed intake by transmission of information to the central nervous system via hepatic vagal afferents (Forbes, 1995; Allen et al., 2009). Animals fed chitosan probably had dry matter intake limited earlier than animals fed other diets due to the increase in DM digestibility and consequently a higher rate of oxidizable fuels reaching the liver. Among the fuels derived from the diet, propionate is most likely to promote oxidation during meals, mainly when high-concentrate diets are fed, because it can be produced fast and extracted from the blood by the liver, stimulating oxidation of acetyl CoA in the TCA cycle (Allen et al., 2009).

Although Araújo et al. (2015) reported a linear increase in DM, NDF, and CP digestibility when evaluating the dose effect of chitosan on the digestion of Nellore steers, the authors did not report differences in DM intake. The authors suggested that those effects were related to altered ruminal fermentation, especially by increasing the propionate concentration. Goiri et al. (2010) also reported that chitosan altered the ruminal fermentation pattern of sheep by increasing propionate proportion and decreasing the acetate to propionate ratio, without effects on DM intake.

Animals fed WS had a lower intake of NFC and increased intake of ether extract. Frequently, when supplemental fat is added to the diet, a source of NFC is withdrawn, and thus the EE content increases and the NFC content of the diet decreases. As there was no effect on DMI when cows received treatment WS, heifers increased their EE intake and decreased their NFC intake.

The results of nitrogen balance suggest better nitrogen utilization, due to greater retained nitrogen when animals were fed diets containing WS compared with CO. Whole raw soybeans partially replaced soybean meal; thus, the protein profile of CO and WS differed in rumen degradable protein values. However, no differences were found in microbial protein flow when cows were fed diets containing WS. High-concentrate diets may affect the efficiency of microbial protein synthesis due to decrease in ruminal pH (Strobel and Russell, 1986). Thus, the results of retained nitrogen may be related to the energy balance, which increased when cows were fed WS. The excess nitrogen in the blood of cows fed CHI or CO was excreted in urine because their net energy intake was lower than that of cows fed WS. VandeHaar (1998) proposed that dietary protein to energy ratios are an important factor in replacement-heifer diets, because an increase in dietary energy density may accelerate heifer growth, leading to an increase in the body protein deposition rate.

Chitosan decreased microbial protein synthesis, and this fact can be associated with its antimicrobial activity. Chitosan exerts greater bactericidal effects against gram-positive than gram-negative bacteria, and antimicrobial activity is enhanced at low pH values (Senel and McClure, 2004). The positive charges of chitosan influence the negative charges of the bacterial cell surface, due to competition with Ca^{+} for electronegative sites on the membrane without conferring dimensional stability, rendering the membrane leaky (Begin and Calsteren, 1999). Increased propionate production is partially explained by the replacement with gram-negative instead of gram positive bacteria (Russel, 1987).

Monensin in several studies reduced ruminal protein degradation and consequently decreased microbial protein flow to the small intestine (Poos et al., 1979; Bergen and Bates, 1984). The decrease in ruminal ammonia production when monensin is supplied can be attributed to inhibitory effects on hyper-ammonia-producing bacteria (Eschenlauer et al., 2002) which have peptidase and deaminase activities (Wallace et al., 1997). Chitosan may have the same effect of monensin in ruminal protein degradation. Furthermore, CHI decreased blood urea concentrations and increased blood creatinine concentrations. The decreased blood concentration of urea can be related to altered ruminal protein degradation, which can reduce the production of ammonia and consequently decrease its absorption and liver metabolism to produce urea.

Creatinine excretion is not greatly affected by changes in diet, and variations in the daily creatinine excretion may be different according to the growth rate of animals (Chizzotti et al., 2008). Creatinine is raised from the muscle metabolism trough the clearance of creatinine phosphate (Harper et al., 1982). Thus, the increase in creatinine excretion by animals fed CHI is related to their higher live weight gain as compared with those fed CO (875.0 and 560.0 g d^{-1}, respectively, data not shown).

Chitosan decreased and WS increased total cholesterol of heifers. Fat supplementation increases lipoprotein cholesterol export by the intestine, the major site of cholesterol synthesis in ruminants (Noble, 1981). Cônsolo et al. (2015) fed increasing doses of WS to Nellore bulls and found a linear increase in total cholesterol and no difference

in glucose concentrations. The mechanism by which CHI alters the cholesterol metabolism is unclear, but studies in humans have demonstrated that chitosan reduced serum LDL cholesterol (Yihua and Binglin, 1997; Wuolijoki et al., 1999), and Bokura and Kobayashi (2003) suggested a reduced lipid absorption from the gastrointestinal tract. However, the difference between EE digestion between CO and CHI was only 13 g kg^{-1} in the current study.

Conclusions

Chitosan improves nutrient digestion and decreases dry matter intake and consequently reduces nitrogen excreted in feces. Whole raw soybean positively affects the energy intake and nitrogen utilization, compared with control or chitosan. Chitosan and whole raw soybeans do not alter nutrient intake and total tract digestion; however, they decrease nitrogen urinary excretion and increase blood HDL cholesterol of heifers.

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