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Effects of Chitosan and Whole Raw Soybeans on Feeding Behavior and Heat Losses of Jersey Heifers

Research Article

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ABSTRACT

This study aimed to determine the effects of chitosan, whole raw soybeans or their interaction on feeding behavior and heat losses through thermography assay of Jersey heifers fed high concentrate diets. Twelve Jersey heifers (age of 6±0.5 months and 139.50±25.56 kg of live weight, mean±SD) were randomly assigned to a replicated latin square design with 2 × 2 factorial treatment arrangement. The experimental period consisted of 14 days of adaptation to the diets, 6 days of sampling and 5 days of wash out. The diets were: control (CO), chitosan (CHI, inclusion of 20 g/kg dry matter (DM) of chitosan), whole raw soybeans (WS, 163.0 g/kg of WS on diet DM basis), and chitosan + whole raw soybeans (CHI+WS). Chitosan decreased DM and neutral detergent fiber (NDF) intake (0.79 and 0.31 kg/d, respectively), increased the eating time (31.88 min) and decreased the NDF content of regurgitate rumen bolus (57 g). Whole raw soybeans did not affect feeding behavior, except for a higher time in standing rest. The association of CHI and WS increased the time which animal ruminated stand. The diets did not influence superficial temperature of heifers. However, WS diet increased heat losses by radiation and convection. The highest values of heat losses were observed after 2 hours of feeding. The interaction of CHI and WS did not alter feeding behavior and heat losses. Feeding WS to heifers increased the total heat losses.

KEY WORDS chitin, heat production, infrared imaging, oilseed, thermography.

INTRODUCTION

Nutritional strategies to improve animal performance and decrease feeding costs are necessary for profitability of heifer production. The dietary addition of whole raw soybean (WS) may decrease feeding costs, since it does not pass by an industrial processing, and it increases diet energy density. Furthermore, the lipid fraction contained in the whole raw soybeans (WS) is slowly released in ruminal environment due to the protein complex that protects the oil contained in cotyledon of seeds, thus not impairing the ruminal fiber digestion (Barletta *et al.* 2016). Chitosan (CHI) is the second most abundant biopolymer in the nature, obtained by the partially deacetylation of chitin (major component of crustacean exoskeleton), and recognized by its antimicrobial properties (Senel and McClure, 2004). Chitosan has been extensively studied during the last decade and has increased the ruminal propionate production (Paiva *et al.* 2016; Araújo *et al.* 2015) and improved the energetic status of dairy heifers (Gandra *et al.* 2016). The production of replacement heifers is a critical and can interfere with the genetic potential for milk production of a dairy herd, but it still an obstacle for the farmers. In addition, behavioral and metabolic tools to adjust diet formulation to dairy heifers in tropical conditions are underused (Oliveira and Ferreira, 2016). The study of the feeding behavior is an important tool of diet evaluation, allowing adjusts of alimentary handling for attainment of better productive performance. The utilization of infrared thermography to monitoring the heifer heat losses may be an important tool to perform dietary adjustments and alleviate the heat stress in tropical conditions. The objective of this experiment was to determine the effects of dietary inclusion of WS and CHI on feeding behavior and heat losses of dairy heifers. Our hypothesis was that feeding both WS and CHI would improve energetic status of dairy heifers in tropical conditions.

MATERIALS AND METHODS

Animals and experimental design

This study was approved by the Bioethics Committee of the Federal University of Grande Dourados. The experiment was conducted at the Animal Science Sector of Federal University of Grande Dourados (UFGD), Dourados, Brazil. Twelve Jersey heifers (age of 6±0.5 months and 139.50±25.56 kg of live weight, mean±SD) were randomly assigned to a replicated Latin square, balanced and contemporaneous, with 2 × 2 factorial treatment arrangement design. The experimental period consisted of 14 days of adaptation to diets, 6 days of sampling and 5 days of wash out. Animals were allocated in individual pens of 8 m^2, containing feed bunks and free access to water.

The experimental diets were: control (CON), CHI (inclusion of 20 g/kg DM of chitosan), WS (163.0 g/kg of WS on diet DM basis), and CHI + WS. Diets were formulated to achieve an average daily gain of 700.0 g/d according to NRC (2001), were isonitrogenous and corn silage was used as the forage source (Table 1). Chitosan had the technical specifications: apparent density of 0.64 g/mL, 20 g/kg of ash, 7.0-9.0 of pH, viscosity < 200 cPs and deacetylation level of 95% (PolymarIndustria e Cia. Imp. And Exp. LTDA, Ceara, Brazil).

Diets were fed as a total mixed ration twice daily (06:30 and 13:00).

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 of total daily orts) from each heifer were collected daily during the last 6 days of each period and combined into one composite sample of ort for each cow and one composite sample of silage. Chemical analyses and estimation of non-fiber carbohydrate, total digestible nutrient and net energy of samples are described in Gandra *et al*. (2016).

Temperature and humidity index (THI) were calculated according to the equation: THI= (9/5 temperature ˚C+32) – (11/2–11/2×humidity) × (9/5 temperature ˚C–26), (Table 2). Heat stress was classified according to some studies, in which: stress threshold is between 68 and 72, mild-moderate stress is between 72 and 79, moderate-severe stress is between 80 and 89, and severe stress is between 90 and 98.

Table 1 Ingredients and chemical composition of experimental diets

Item	Diet			
	CON	CHI	WS	CHI + WS
Ingredient (g/kg 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[1]	51.1	51.1	51.1	51.1
Chitosan	-	2.00	-	2.00
Chemical composition (g/kg DM)				
Dry matter	573.0	573.0	575.5	575.5
Crude protein (CP)	149.5	149.5	149.0	149.0
Ether extract (EE)	24.8	24.8	72.0	72.0
Neutral detergent fiber (NDF)	378.3	378.3	383.8	383.8
Non-fiber carbohydrate (NFC)[2]	397.7	397.7	336.9	336.9
Ash	49.3	49.3	51.4	51.4
Total digestible nutrient	710.0	710.0	774.3	774.3
Net energy[3] (Mcal/d)	1.62	1.62	1.78	1.78
Net energy for gain[3] (Mcal/d)	1.20	1.20	1.39	1.39

CON: control; CHI: chitosan, addition of 20 g/kg diet DM of chitosan; WS: whole raw soybeans, diet containing 7.2% EE on diet DM basis and CHI + WS: chitosan and whole raw soybeans, addition of 20 g/kg diet DM of chitosan and diet containing 7.2% EE.
[1] Contained per kilogram: Ca: 120 g; P: 88 g; I: 75 mg; Mn: 1300 mg; Na: 126 g; Se: 15 mg; S: 12 mg; Co: 3630 mg; Cu: 55.50 mg and Fe: 1800 mg.
[2] NFC= 100 - [(% CP-% CP from urea+% urea) + % EE + % ash + % NDF].
[3] Calculated according to NRC (2001) model.

Table 2 Environmental temperature and humidity during the first eight hours after feeding time

Item	Temperature (˚C)	Humidity (%)	THI*
0 hour			
Minimum	23.20	49.00	69.59
Average	25.37	57.50	72.19
Maximum	27.00	74.00	77.36
2 hours			
Minimum	23.10	51.00	69.46
Average	24.67	58.75	73.78
Maximum	28.30	79.00	76.49
4 hours			
Minimum	25.40	39.00	71.56
Average	28.90	50.00	76.90
Maximum	33.40	68.00	82.85
6 hours			
Minimum	28.20	29.00	73.63
Average	31.17	42.50	78.54
Maximum	36.80	58.00	84.07
8 hours			
Minimum	30.30	35.00	76.92
Average	33.17	42.00	80.95
Maximum	36.40	50.00	85.60

THI= (9/5 temperature ˚C+32) – (11/2–11/2×humidity) × (9/5 temperature °C–26).

Feeding behavior

All animals were submitted to a 24-hour period of visual observation for evaluation of the feeding behavior. The data collection of time spending in feeding, rumination and idleness activities was performed on day 20 of each period every five minutes using a digital camera with night vision (PRO-510 CAM, Swann, Victoria, Australia) handled by one observer during the period. The determination of the number of cud chews and time spent in the rumination of each ruminal bolus were assessed on the following day using a digital chronometer.

Three ruminal boluses of each animal of the experiment were evaluated by observation, in three different periods of the day (between 10:00 and 12:00; from 14:00 to 16:000; and between 19:00 and 21:00). The environment was maintained with artificial illumination during the night observation of animals (Costa *et al.* 2014). The number of ruminal boluses, chewing time, ruminating time, and the eating, chewing and rumination efficiencies were obtained according to Bürger *et al.* (2000).

Infrared thermal images and heat loss

Infrared thermal images were performed on days 15, 16 and 17 of each experimental period before (time 0) and 2, 4, 6 and 8 hours after the morning feeding using a thermal camera (Testo 880, Brandt Instruments, Prairieville, LA, USA). The anatomical regions assessed by thermal camera were: left and right flanks, rump and head (Martello *et al.* 2009); (Figure 1).

The emissivity value used was 0.98 and images were recorded from approximately 1.5 m of the animals (Gomes *et al.* 2016). Total sensible heat loss (Q) was calculated as function of heat loss by radiation (Qr) and by convection (Qc), as suggested by Yahav *et al.* (2004) and Van Brecht *et al.* (2005), respectively.

(1) $Q= Qr + Qc$
(2) $Qr= \varepsilon\sigma A\ (Ts^4-Tair^4)$
(3) $Qc= hA\ (Ts-Tair)$
(4) $h= 0.336 \times 4.184 \times (1.46+\sqrt{V_{air}}-100)$

Where:
Q: total sensible heat (W).
e: heifer emissivity (0.98).
σ: Stefan-Boltzman constant ($5.67m^{-2}\times10^{-8}$, W m^{-2} K^{-4}).
A: heifer surface area (m^2).
h: heat transfer coefficient given by Eq. 4 (15 W m^{-2}), V_{air}= air velocity, Qr= heat loss by radiation (W), Qc= heat loss by convection (W), Ts= heifer's surface temperature (˚C), and Tair= air temperature (˚C). The area (A, m^2) in Eq. 2 and Eq. 3 was estimated as the average area of a spherical form exposed to convective and radiant heat transfer.

Statistical analyses

Data were submitted to analysis of variance using the PROC MIXED (SAS, 2004) verifying the normality of residuals and homogeneity of variances using PROC UNIVARIATE, according to the following model:

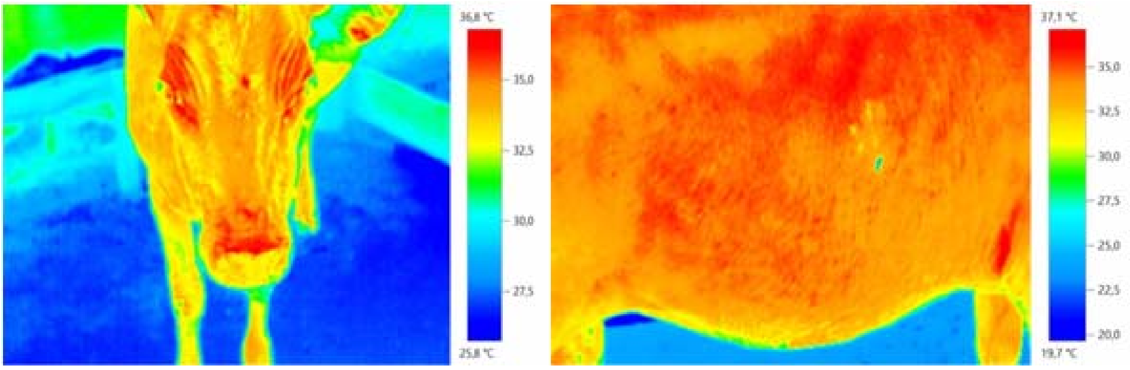

Figure 1 Examples of infrared thermal images of Jersey heifers

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

Where:
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 by whole raw soybean interaction fixed effect.
P_jC_k: period by chitosan interaction fixed effect.
P_jW_k: period by whole raw soybean interaction fixed effect.
e_{ijkl}: residual error.

The degrees of freedom were calculated by DDFM= k × r. Significance level was set at 0.05. PDIFF test was applied when interaction effect was observed to determine differences among treatments. Data of infrared thermal images and heat losses were submitted to MIXED procedure adding to the model the fixed effect of time (hours) in relation to the feeding, and it interaction with treatments, also as fixed effect.

RESULTS AND DISCUSSION

Heifers were submitted to mild-moderate stress until 2 hours after feeding, and moderate-severe stress from 4 hours until 8 hours after feeding according to the maximum calculated THI values (Table 2). Chitosan decreased DM (P=0.041) and NDF (P=0.014) intake (Table 3). However, animals fed CHI showed longer (P=0.003) eating time than CON, CHI and CHI + WS.

Interaction effect (P=0.023) was observed on standing and ruminating which was higher when heifers were fed chitosan associated with supplemental fat compared to CON or CHI, but did not differ of animals fed WS. Moreover, WS increased standing rest period (P=0.020). Chitosan decreased (P=0.043) neutral detergent fiber on regurgitate rumen bolus (Table 3).

Chitosan decreased DM (P=0.009) and NDF (P=0.004) eating efficiency. Likewise heifers fed CHI showed lower NDF (P=0.019) chewing efficiency compared to the other treatments. Interaction effect (P=0.007) was observed on DM rumination efficiency which was lower when heifers were fed chitosan associated with supplemental fat compared to CON or CHI, but did not differ of animals fed WS (Table 3). Infrared thermal images from left and right flanks, hump and head of heifers were not altered by treatments (Table 4). Moreover, heifers fed WS showed lower heat losses by radiation (P=0.036), convection (P=0.035), and total heat losses (P=0.008) compared to the other treatments. Time effect was observed on heat losses by radiation (P=0.012), convection (P=0.003), and total heat losses (P=0.021). Heifers fed WS showed lower heat losses by radiation at 4 and 8 hours after feeding, for convection at 0, 2 and 8 hours after feeding and total losses at 0, 2, 4, 6 and 8 hours after feeding (Figures 2, 3 and 4).

Several studies reported no differences of DM intake when CHI was supplied to ruminants (Goiri *et al.* 2010; Araújo *et al.* 2015), however both studies reported increase of DM total tract digestion with CHI dietary addition. The decreased DM intake of heifers may be related to the higher DM total tract digestion in animals fed CHI compared to CON (0.692 *vs.* 0.677 g/kg, respectively; Gandra *et al.* 2016), thus more nutrient would be absorbed by the intestine. Oxidizable fuels reaching the liver can affect feed intake by transmitting information to the central nervous system, interrupting the meal (Allen *et al.* 2009). In addition, ruminants supplemented with CHI showed increase of propionate production in rumen (Araújo *et al.* 2015) which decreases the feed intake (Allen, 2000). The reduction of NDF intake by animals fed CHI is related to the DM intake. The increase of eating period of heifers fed CHI may be related with the metabolic regulation of feed intake. High concentration of ruminal propionate increases the satiety level in ruminants, which reduces the length of a meal but increase the number of meals in a manner that increase the time spend eating.

Table 3 Effects of chitosan and whole raw soybeans on dry matter intake and feeding behavior of Jersey heifers

Item	Diet				P-value		
	CON	CHI	WS	CHI + WS	CHI	WS	INT
Intake (kg/day)							
Dry matter	6.45±0.18	5.66±0.19	5.97±0.21	5.86±0.21	0.041	0.260	0.262
Neutral detergent fiber	2.21±0.06	1.90±0.06	2.05±0.08	1.92±0.08	0.014	0.425	0.288
Length of periods (min)							
Eating	343±3.97	375±3,98	339±4.01	345±3.95	0.003	0.215	0.331
Chewing	619±7.02	630±7.07	606±7.09	614±6.98	0.532	0.329	0.889
Ruminating	484±6.09	473±6.07	500±6.14	495±6.11	0.736	0.435	0.910
Standing and ruminating	95.6[b]±5.08	88.1[bc]±5.32	114[ab]±5.12	129[a]±5.12	0.646	0.556	0.023
Lying and ruminanting	388±4.08	384±4.12	386±4.11	366±4.12	0.575	0.635	0.635
Resting	439±5.09	456±5.32	431±5.32	412±5.31	0.974	0.398	0.550
Standing rest	99.4±3.09	105±3.03	137±3.02	126±3.01	0.415	0.020	0.442
Lying rest	339±4.08	330±4.07	294±4.06	308±4.04	0.930	0.126	0.599
Regurgited rumen bolus							
Number	537±7.12	540±7.13	550±7,14	533±7.10	0.945	0.229	0.684
Time (sec)	56.0±1.28	50.5±1.33	52.9±1.32	55.5±1.30	0.668	0.779	0.233
Mastication (n)	50.5±0.66	52.9±0.54	54.0±0.72	53.1±0.70	0.549	0.144	0.203
Dry matter (g)	12.1±0.44	10.9±0.34	10.9±0.40	10.9±0.38	0.206	0.274	0.174
Neutral detergent fiber (g)	4.13±0.10	3.56±0.12	3.76±0.13	3.58±0.14	0.043	0.323	0.261
Efficiency							
Eating (g/h)							
Dry matter	1144±6.10	991±6.12	1118±6.12	1040±6.10	0.004	0.451	0.470
Neutral detergent fiber	391±3.44	321±3.39	383±3.38	341±3.41	0.009	0.754	0.754
Chewing (g/h)							
Dry matter	626±4.95	563±4.98	590±4.95	577±4.97	0.564	0.561	0.702
Neutral detergent fiber	214±3.67	183±3.55	203±3.62	189±3.55	0.019	0.788	0.306
Rumination (g/h)							
Dry matter	845[a]±4.50	786[a]±4.55	734[ab]±4.67	721[b]±4.55	0.478	0.097	0.007
Neutral detergent fiber	290±3.55	253±3.51	253±3.56	237±3.54	0.149	0.150	0.550

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
CON: control; CHI: chitosan, addition of 20 g/kg diet DM of chitosan; WS: whole raw soybeans, diet containing 7.2% EE on diet DM basis and CHI + WS: chitosan and whole raw soybeans, addition of 20 g/kg diet DM of chitosan and diet containing 7.2% EE.
CHI: chitosan effect; WS: whole raw soybean effect and INT: interaction effect of CHI + WS.

Table 4 Effect of chitosan and whole raw soybeans superficial temperature and heat losses of Jersey heifers

Item	Diet				P-value		
	CON	CHI	WS	CHI+WS	CHI	WS	INT
Superficial temperature (˚C)							
Left flank	34.55±0.15	34.59±0.15	34.52±0.18	34.30±0.17	0.563	0.325	0.414
Right flank	34.65±0.22	34.44±0.21	34.50±0.20	34.39±0.20	0.554	0.337	0.770
Rump	34.31±0.14	34.29±0.15	34.32±0.17	34.27±0.18	0.839	0.982	0.946
Head	34.13±0.17	33.87±0.15	33.99±0.14	34.03±10.19	0.516	0.950	0.357
Heat losses (w/m^2)							
Radiation	0.049±0.01	0.051±0.02	0.045±0.01	0.047±0.01	0.888	0.036	0.471
Convection	64.17±1.14	65.48±1.17	62.06±1.12	71.95±1.12	0.311	0.035	0.692
Total	64.22±1.32	65.53±1.31	62.11±1.33	72.00±1.29	0.564	0.008	0.750

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
CON: control; CHI: chitosan, addition of 20 g/kg diet DM of chitosan; WS: whole raw soybeans, diet containing 7.2% EE on diet DM basis and CHI + WS: chitosan and whole raw soybeans, addition of 20 g/kg diet DM of chitosan and diet containing 7.2% EE.
CHI: chitosan effect; WS: whole raw soybean effect and INT: interaction effect of CHI + WS.

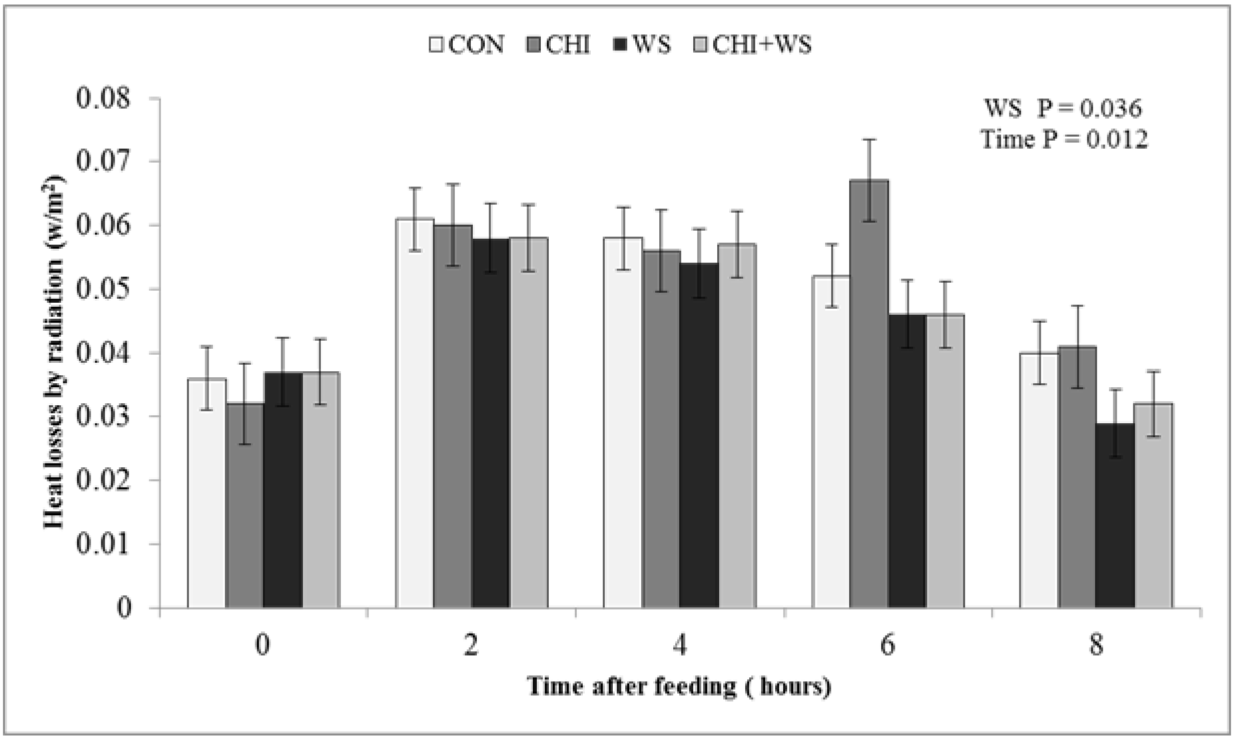

Figure 2 Effects of chitosan and whole raw soybeans on heat losses by radiation according to the time after feeding
CON: control; CHI: chitosan, addition of 20 g/kg diet DM of chitosan; WS: whole raw soybeans, diet containing 7.2% EE on diet DM basis and CHI + WS: chitosan and whole raw soybeans, addition of 20 g/kg diet DM of chitosan and diet containing 7.2% EE

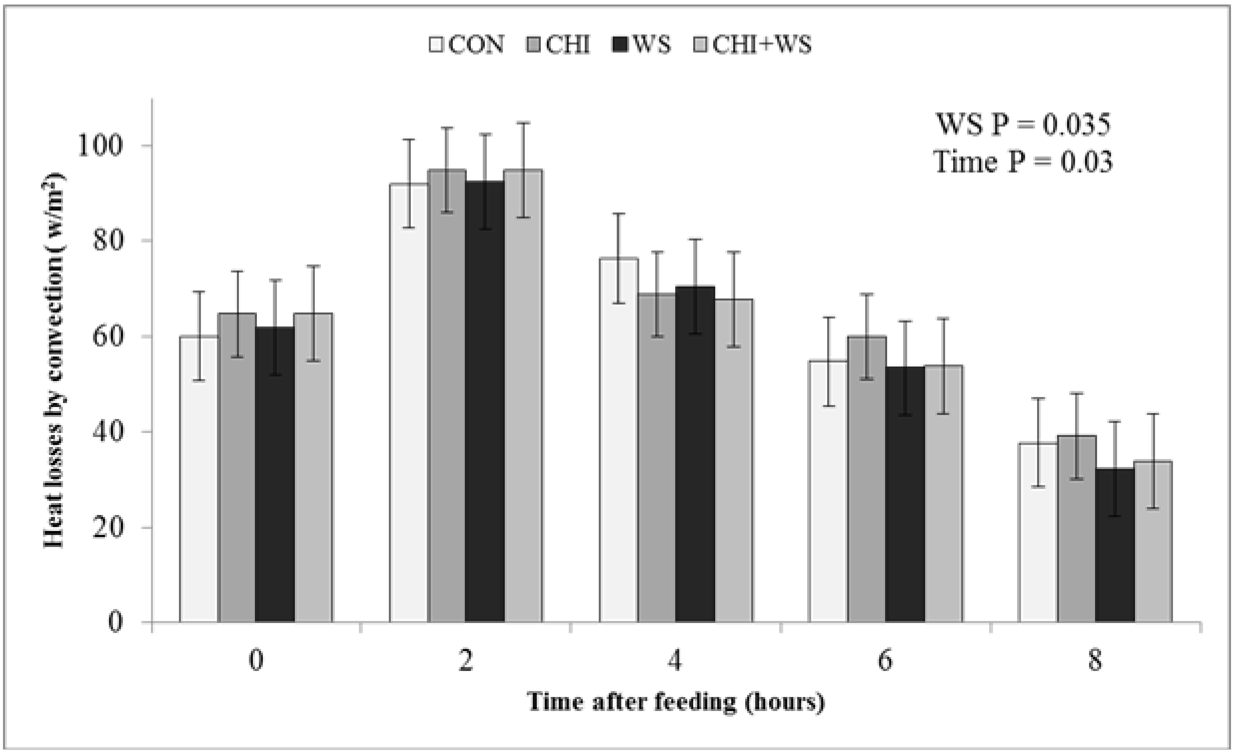

Figure 3 Effects of chitosan and whole raw soybeans on heat losses by convection according to the time after feeding
CON: control; CHI: chitosan, addition of 20 g/kg diet DM of chitosan; WS: whole raw soybeans, diet containing 7.2% EE on diet DM basis and CHI + WS: chitosan and whole raw soybeans, addition of 20 g/kg diet DM of chitosan and diet containing 7.2% EE

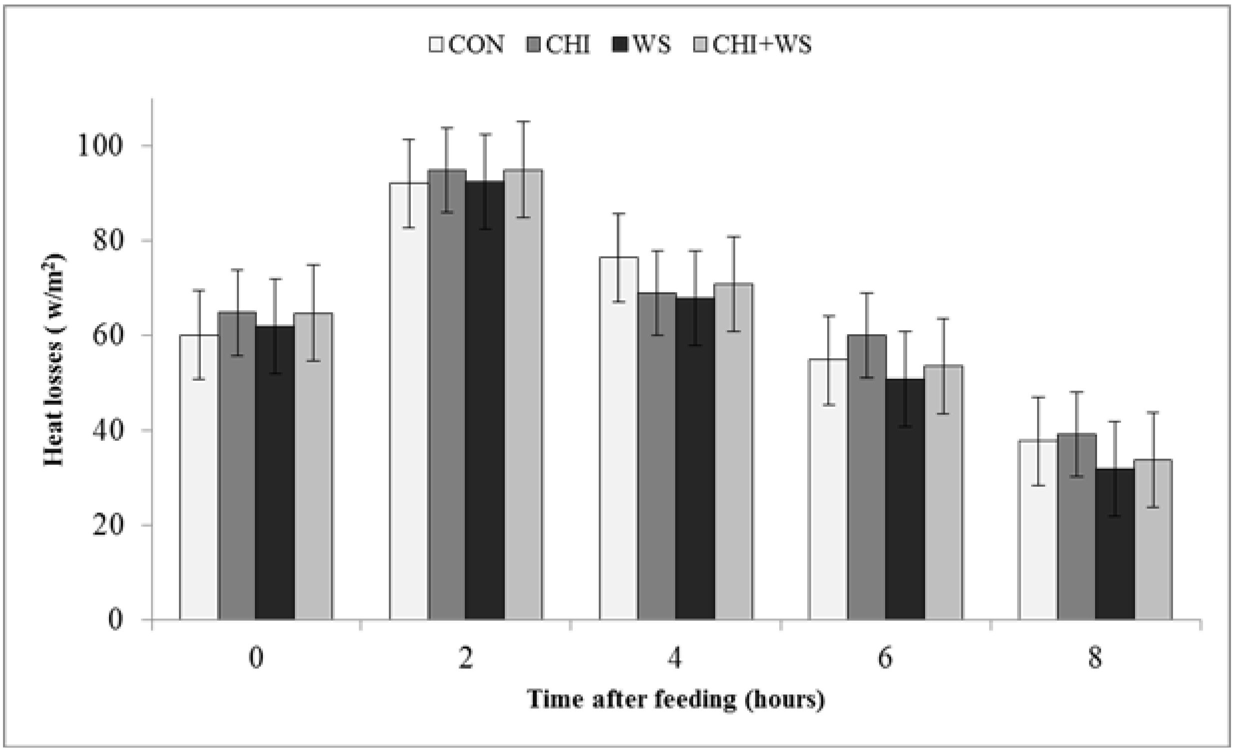

Figure 4 Effects of chitosan and whole raw soybeans on total heat losses according to the time after feeding
CON: control; CHI: chitosan, addition of 20 g/kg diet DM of chitosan; WS: whole raw soybeans, diet containing 7.2% EE on diet DM basis and CHI + WS: chitosan and whole raw soybeans, addition of 20 g/kg diet DM of chitosan and diet containing 7.2% EE

In addition, the values of the time spend eating reported in the current experiment agree with several studies (DeVries *et al.* 2009; Greter *et al.* 2010; Huzzey *et al.* 2013).

The eating efficiency of DM and NDF, and chewing efficiency of NDF were reduced in heifers fed CHI due to the lower DM intake associated with the lower spend time ruminating stand compared to other treatments. Since that a reduction of ruminating efficiency cannot be compensated by an increase of the time spend ruminating, the efficiency of ruminating is important to control the utilization of roughage and restrict the utilization of low quality feed ingredients which comprise the productive performance of animals (Huzzey *et al.* 2013).

The increase of ruminating efficiency of DM showed by the heifers fed CHI is related to the ether extracts (EE) dietary content of other diets, because high levels of EE may contribute to the reduction of ruminating efficiency of DM. Another fact that may contribute to the increase of ruminating efficiency when heifers were fed CHI is the absence of whole soybean grains in diet which could reduce the particle size of regurgitate digesta, and thus, increasing the ruminating efficiency of DM (Dulphy *et al.* 1980; Silva *et al.* 2005).

Heat stress results from the animal's inability to dissipate sufficient heat to maintain homeothermy. Environmental factors, including ambient temperature, radiant energy, relative humidity, and metabolic heat associated with maintenance and productive processes, contribute to heat stress (West, 2003). The superficial temperatures measured using infrared thermography on left and right flanks, head and rump agree with the data reported by other studies (Kotrba *et al.* 2007; Montanholi *et al.* 2008). The skin temperature reflects heat dissipation (Scharf *et al.* 2010). Non-evaporative heat losses are determined by the animal to environment temperature gradient and by the amount of body surface area (Berman, 2003). Thus, the thermoregulatory strategy of an animal, based on the assumption of stable deep body temperature, should be aimed at minimizing the gradient between their coat surface temperature and the temperature of the environment, since this will greatly reduce the flow of heat (Gomes *et al.* 2016).

The lower heat losses by radiation, convection and total heat losses observed in heifers fed WS can be explained by the EE dietary content (72.0 g/kg). The fat addition in dairy cow diets could decrease the heat load of dairy cows because of the high energy density and lower metabolic heat when compared with other ingredients such as fiber and carbohydrate (Morrison, 1983). Fats are not digested in the rumen so production of heat in the rumen from fat digestion is minimal. Therefore; internal heat produced per unit of energy consumed should be less for cows supplemented with fat. Total heat loss was reduced by 4.9 and 7.0% when cows were fed whole cottonseed at 15% of dietary DM or whole seed plus 2.64 kg/d of calcium salts of palm oil distillate (Holter *et al*. 1992).

The heat losses in relation to the morning feeding show that animals constantly seek for the homeostasis. Although, the environmental factors, diet formulation and physiological events of digestion demonstrate that animals were submitted to heat stress over approximately 87.8% of experimental period. Thermostasis is the process by which cows attempt to keep their body temperature constant in spite of changes in environmental temperatures. Heat stress occurs when the cow is incapable of dissipating enough heat to maintain its core body temperature below 38.8 ˚C (Martello *et al*. 2009). This increase in body temperature results from the combination of heat from the environment and that produced internally during rumen fermentation and nutrient metabolism (Drackley *et al*. 2003).

CONCLUSION

Chitosan decreased DM and NDF intake, altering the time in which animals spend eating and chewing. The association of CHI and WS increased the period in which heifer ruminated stand and decreased the rumination efficiency of DM. Chitosan did not affect body surface temperature and heat losses. Whole raw soybeans decreased the total heat losses of animals. The association of CHI and WS did not positively influence the feeding behavior and heat losses of dairy heifers.

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