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Effect of natural feed additives on meat quality and caecotrophic fatty acid profile of New Zealand rabbits

Efeito de aditivos alimentares naturais na qualidade de carne e no perfil de ácidos graxos cecotrófico de coelhos Nova Zelândia

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ABSTRACT

The objectives of this research were to evaluate the effects of commercial probiotic and chitosan as food additives on the quality and meat composition of 36 New Zealand White rabbits (57 ± 8 days old and 1,648 ± 0.194 kg) and on the fatty acid profile of caecotrophs. The treatments were CT (diets without inclusion of additives), PRO (inclusion of 4 g / kg of commercial probiotic) and CHI (inclusion of 4 g / kg of chitosan). The additives increased triglycerides and decreased urea compared to the control group, as well as increased oleic and linoleic acids, Σ unsaturated, Σ monounsaturated and Σ polyunsaturated in caecotrophs. CHI animals showed a decrease in myristic and palmitic acids compared to PRO. CHI decreased the meat's crude protein and the meat's fat. In addition, there was a decrease in omega-3, omega-6 and the relationship unsaturated and saturated fatty acids for the CHI group and an increase in erucic acid and a decrease in the rate of hypocholesterolemic acids. As a conclusion, the data showed that the animals that ingested probiotic had better meat quality, for having better fatty acid profile and hypocholesterolemic index, compared to the treatment with chitosan. The additives improved the caecotrophs fatty acid profile.

Keywords: chitosan, digestive health, microbial additive, probiotic, triglyceride

RESUMO

Os objetivos desta pesquisa foram avaliar os efeitos do probiótico e da quitosana como aditivos alimentares na qualidade e composição da carne de 36 coelhos da raça Nova Zelândia Branco (57±8 dias de idade e 1.648±0.194 kg) e no perfil de ácidos graxos dos cecotrofos. Os tratamentos foram CT (dietas sem inclusão de aditivos), PRO (inclusão de 4 g/kg de probiótico) e CHI (inclusão de 4 g/kg de quitosana). Os aditivos aumentaram os triglicerídeos e diminuíram a ureia em comparação ao grupo controle, bem como amentaram os ácidos oleico e linoleico, Σ insaturados, Σ monoinsaturados e Σ poli-insaturados nos cecotrofos. Os animais CHI apresentaram diminuição nos ácidos mirístico e palmítico em comparação ao PRO. A CHI diminuiu a proteína bruta da carne e o extrato etéreo da carne. Além disso, houve uma diminuição no ômega-3, ômega-6 e a relação entre ácidos graxos insaturados e saturados para o grupo CHI e aumento do ácido erúcico e diminuição do índice de hipocolesterolemia. Como conclusão, os dados mostraram que os animais que ingeriram probiótico apresentaram melhor qualidade da carne, por apresentarem melhores perfil de ácidos graxos e índice hipocolesterolêmico, em comparação ao tratamento com quitosana. Os aditivos melhoraram o perfil de ácidos graxos dos cecotrofos.

Palavras-chave: aditivo microbiano, probiótico, quitosana, saúde digestiva, triglicerídeo

INTRODUCTION

In order to attempt the demand for the modern consumers' desire for a healthy lifestyle, rabbit meat can be an interesting source of high contents of polyunsaturated fatty acids, proteins, and essential amino acids (Li et al., 2018). Rabbit meat products have been rapidly developed during the last two decades, with an increased on the global rabbit meat production by 16% between 2010-2015 (FAOSTAT, 2015), and have become increasingly popular worldwide (Li et al., 2018).

To maximize the animal's growth some antibiotics has been use as growth promoters, it can improve feed efficiency, muscle growth and carcass weight (Falcão-e-Cunha et al., 2007). However, concerns were raised that the use of antibiotics for growth promotion could lead to a problem of increasing resistance in bacteria of human and animal origin, and risks to human health (Falcão-e-Cunha et al., 2007; Yang et al., 2019). As consequence, there has been considerable effort by the scientific community and the animal feed industry to find alternatives to conventional antibiotics in meat production. In this sense, the investigation of natural alternatives free residues, such as probiotics and chitosan, has become important on animal production.

The probiotics has been largely reported in cattle, chicken and pigs (Ouwehand et al., 1999; Abdou et al., 2018). Generally, the probiotics mechanisms of action are reduction of metabolic reactions which produce toxic substances, stimulation of host enzymes, production of vitamins or antimicrobial substances, competition for adhesion to epithelial cells and an increased resistance to colonization, and stimulation of the immune system of the host (Falcão-e-Cunha et al., 2007)

Chitosan is one of the most abundant natural polysaccharide biopolymers. Over the few years, chitosan has

received much attention due to its potential antimicrobial properties against bacteria, fungi, and yeasts (Raafat & Sahl, 2009). Also, chitosan might alter fermentation at rumen and cecum for more energetically efficient patterns and may provide an alternative to antimicrobial growth promoters (Goiri et al., 2010; Araújo et al., 2015; Paiva et al., 2015). However, little had been done incorporate chitosan and probiotic as natural growth promoter in New Zealand white rabbits. Therefore, the aim of this study was to evaluate the effects of probiotic and chitosan addition to the diets on meat quality and caecotroph fatty acids profile of New Zealand white rabbits.

MATERIAL AND METHODS

All animal procedures used in this study were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines of the Federal University of Grande Dourados and approved by the animal ethics committee (Protocol Number: 034/2107).

The feedlot study was conducted in Dourados, Brazilian Center-West region (latitude 22º13'18.54" South, longitude 54°48'23.09" West and an average altitude of 430 m). The experiment lasted for 7 wks. which included 1 wk. adaptation period and 6 wks. experimental period.

Thirty-six New Zealand White rabbit male with 57±8 days age and an average body weight (ABW) of 1.648 kg ± 0.194 were housed in individual cages (40 cm length x 60 cm width x 45 height) with *ad libitum* access to feed and water. During the trials, rabbits were housed in a ventilated building in which the maximum temperature was 25°C and the relative humidity ranges from 50% to 60%. A cycle of 12 h of light and 12 h of dark was used throughout this trial. The diet used was a commercial formulate based on alfalfa hay, soybean meal, and mineral premix, with 18% of crude protein, 12% of crude fiber and 2.6 Kcal/g of digestible energy (Table 1). The diameter of the pellets was 4 mm.

Table 1. Chemical composition of the commercial basal diet offered to rabbits

Nutrient	Content
Dry matter	870.0 g/kg
Crude protein	140.00 g/kg
Organic matter	850.0 g/kg
Fat	30 g/kg
Crude Fiber	260.0 g/kg
Neutral detergente fiber	225.0 g/kg
Acid detergente fiber	180.0 g/kg
Ash	150.0 g/kg
Calcium	15.0 g/kg
Phosphor	6.0 g/kg
Sodium	2.2 g/kg
Copper	15.00 mg/kg
Manganese	40.00 mg/kg
Zinc	65.00 mg/kg
Cobalt	1.00 mg/kg
Selenium	1.00 mg/kg
Vitamin A	10.000.00 UI/kg

Vitamin D3	1.2000.00 UI/kg
Vitamin E	20.00 UI/kg
Lysine	6.00 g/kg
Methionine	2.00 g/kg
Fatty acids (g/100g)	
C 16:0	11.35
C 16:1	2.43
C 18:0	5.21
C 18:1	23.31
C 18:2	48.45
C 18:3	6.21

Upon arrival, rabbits were weighed, and distributed in a completely randomized design in three groups according treatments: CT) diets with no additives inclusion; PRO) Inclusion of 4 g/kg of probiotic (Equisflora® Kera Nutrição Animal, Bento Gonçalves-RS; composition: *Bifidobacterium bifidum* $5x10^8$ ufc g^{-1}; *Enterococcus faecium* $5x10^8$ ufc g^{-1}; *Lactobacillus casei* $5x10^8$ ufc g^{-1}; *Pediococcus acidilactici* $5x10^8$ ufc g^{-1}; *Saccharomyces cerevisiae* $10x10^9$ ufc g^{-1}) on diet dry matter (DM); CHI) Inclusion of 4 g/kg of chitosan (Polymar Industria e Cia. Imp. and Exp. Ltda., Ceará, Brazil; technical specifications: density of 0.64 g mL−1, 20 gkg−1 of ash, 7.0-9.0of pH, viscosity <200 cPs, and deacetylation level of 95%) on diet DM. Animals were allocated in individual cages (12 pens per treatment).

Feeds were provided twice daily, at 08:00 h and 14:00 h and the residual feed in the raising cages was collected daily for adjusted the daily offer according to the weight of the leftovers, to allow a minimum of 3 % and a maximum of 5 % of orts. The additives were included in the mineral premix and to the pellet mixture.

Blood samples were collected at the beginning and at the end of additives supplementation by puncture of the aorta vein or artery prior to the morning feeding. Blood samples (10 mL) were collected into 10-mL tubes (BD Vacutainer, São Paulo, SP, Brazil), without anticoagulant, for the measurement of serum glucose, cholesterol, triacylglycerol, total protein, and serum urea. Serum metabolites were analyzed calorimetrically according to standard procedures using commercially available diagnostic kits (Randox Laboratories, São Paulo, SP, Brazil) in an ABS-200 automatic biochemistry analyzer (CELM, São Caetano do Sul, SP, Brazil).

For caecotroph lipid profile, samples of caecotroph were taken at day 15, 30 and 45 of the experimental period. The light plastic collars at 25 cm^2 were put on the animals at 09:00 to prevent caecotroph. Caecotroph sampling was performed at 14:00, 18:00 and 09:00 of the next day (after 24 h), when the collars were removed from the animals. For each rabbit an amount of fresh caecotroph was immediately used to determine DM content and the remaining was freeze-dried.

After 60 days on feedlot, rabbits were electrically stunned (70 V, pulsed direct current, 50 Hz for 5 s) and killed by cervical dislocation. After 24 h postmortem the carcass pH was recorded and samples from *Longissimus lumborum* (LL) were taken for color, water holding capacity (WHC), cooking loss (CL), Warner Bratzler Shear Force (WBSF), and proximate analysis. The

color was measure using a Minolta CR 200b in the L*, a* and b* system. ΔE was calculated to check if there is a difference between the color of the meat according to Technical Report Colorimetry (CIE, 2004), using the formula $\Delta E = ((\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L)^2)^{0.5}$, when Δa, Δb and ΔL are, respectively, the variation between the values of the reddish, yellowish and luminosity variables of the standard and those obtained through the readings of the Minolta equipment. Perceptible difference to the human eye was considered ΔE> 2.3 units, according to Mancini et al. (2019).

The water holding capacity was determined according to Torres (2005). The 3.0 g meat samples was subjected to compression by 2.25 kg standard weight, for five minutes. Meat chemical composition was determined in all samples (triplicate). Meat was thawed over the night before analysis, minced, and homogenized to determine moisture, ash, crude protein (CP), and extract ethereo (EE) (AOAC, 2000).

The WBSF and CL were determined according the methodology proposed by (Wheeler et al., 2005). The LL samples were thawed for 24 h at 4 °C, weighed, and roasted in an oven equipped with a thermostat adjusted to 170 °C (Flexa de Ouro Industry, São Paulo, SP, Brazil). The steaks internal temperature was monitored using individual thermometers (Globo Industry, Americana, SP, Brazil) until it reached 71 °C. The samples were cooled to 28 °C and weighed again, thus obtaining the value for CL. Steaks were cooled at 4 °C for 24 h before shearing. For WBSF evaluation, cores with 1.3 cm of diameter were taken from each steak, parallel to the orientation of the muscle fibers. Each core was sheared perpendicular to the muscle fiber using a WBSF instrument (Warner-Bratzler meat Shear, G-R Manufacturing, Collins, KS, USA), according to standard procedures from American Meat Science Association (AMSA, 1995). The WBSF values of the six subsamples were averaged for statistical analysis.

To proximate analyses, 2 g of *LL* were sampled after 24 hours' postmortem to evaluate DM, ash, CP and EE. The DM was analyzed by the method 950.15 (AOAC, 2000), samples were weighed out into pre-dried, pre-weighed containers and allowed to dry for 18h at 100°C in an air oven. The samples were cooled and weighed to obtained DM value. Ash was evaluated by the method 942.05 (AOAC, 2000). Samples were weighed into pre-dried, pre-weighed crucibles and placed into a specific oven at 550°C for 24h. Samples were cooled in a desiccator and weighed.

Crude protein was determined using the AOAC (2000) N × 6.25; method 984.13. Total nitrogen was determined with a furnace temperature of 1050°C with helium as the carrier gas as previously described by (Wahrmund-Wyle et al., 2000). Crude protein levels were determined by multiplying total nitrogen by a factor of 6.25.

The EE were also analyzed according AOAC (2000) method 920.39. Samples were homogenized with 20 mL chloroform: methanol (2:1) in a 50 mL screw cap polypropylene tube. The homogenate was filtered through a Buchner funnel with slight suction as previously described by Wahrmund-Wyle et al. (2000). The filter was rinsed with chloroform: methanol. The filtrate was transferred back into the 50 mL tube, and 8 mL of a 0.74% KC1 solution was added. After separation, the upper phase was siphoned off, and the lower phase was transferred into pre-dried, pre-weighed beakers.

To determine the meat and caecotroph fatty acid profile, samples were saponified, and the fatty acids were extracted and methylated using the method of Hara & Radin (1978) and Christie (1982). The fatty acid profile was analyzed by gas chromatography (Thermo Finnigan, Trace 2000) using an SP-2560 silica capillary column (100 m × 0.25 mm in diameter with 0.02 mm thickness, Supelco, Bellefonte, PA). One standard (CRM-164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to identify the fatty acids.

Data were submitted to analysis of variance using the PROC MIXED (version 9.3, SAS Institute, Cary, NC), according to the following model:

$$Y_{ij} = \mu + A_i + T_j + e_{ij}$$

with $e_{ijk} \approx N(0, \sigma_i^2)$; where Y_{ijk} is the value of dependent variable, μ is the overall mean, A_i is the fixed effect of animals (i = 1 to 24), T_j is the fixed effect of treatment (j = 1 to 3), e_{ij} is the residual error, N stands for Gaussian distribution, and σ_i^2 is the variance associated with each treatment. Degrees of freedom were corrected by Kenward and Rogers (1997) method. The effect of treatments was decomposed into orthogonal contrasts: (1) control *vs.* additives and (2) chitosan *vs.* probiotic. Other comparisons were made using Fisher's protected LSD. Significance level was set at $P<0.05$ and tendence at $P<0.10$.

RESULTS

Initial body weight (BW) was similar for the treatments, demonstrating homogeneity at the initial allocation (Table 1). The inclusion of feed additives in the diet did not change (contrast 1; $P > 0.05$) final body weight and dry matter intake. According serum metabolites, the additives increased ($P = 0.001$) the triglycerides by 31% and decreased ($P = 0.023$) urea by 6% compared to control group (Table 1). When additives were compared between each other, the chitosan increased ($P = 0.032$) by 30% ($P = 0.032$) and by 21% ($P = 0.013$) the serum glucose and triglycerides, and increased by 8,5% ($P = 0.016$) the cholesterol compared with animals fed PRO diet. Also, there was no effect of treatments ($P > 0.05$) on serum total protein concentration between treatment (Table 2).

Table 2. Effects of probiotic and chitosan on body weight and blood parameters of New Zealand white rabbits.

Item	Diet[A]			SEM	*P-Value*[B]	
	CT	PRO	QUI		C1	C2
Body weight (kg)						
Inicial	1.73	1.62	1.67	0.03	0.425	0.578
Final	2.50	2.64	2.64	0.05	0.273	0.995
Blood parameters						
Glucose (mg/dL)	118.0	129.2	90.0	0.62	0.541	0.032
Cholesterol (mg/dL)	168.5	155.7	169.0	0.88	0.411	0.013
Triacylglycerols (mg/dL)	103.2	151.5	119.5	1.78	0.001	0.016
Total protein (g/dL)	7.90	8.12	8.65	0.45	0.113	0.216
Albumin (g/dL)	3.06	3.22	3.28	0.89	0.247	0.792
Urea (mg/dL)	47.6	43.0	43.8	0.98	0.023	0.628

[A]Diets: CT, control, with no additives inclusion; PRO, diet with 4 g/kg of DM of probiotic inclusion; CHI, diet with 4 g/kg of DM of chitosan inclusion

[B]C1, contrast between CT and Additives; C2, contrast between PRO and CHI.

There were an increased on oleic (P = 0.018) and linoleic acids (P =0.013) in caecotroph from rabbits fed additives compared to the CT group (Table 2), consequently, the Σ unsaturated (P = 0.003), Σ monounsaturated (P = 0.013), and Σ polyunsaturated (P = 0.006) was also increased by additives compared to the CT diet (Table 2). When the CHI and PRO were compared, there was a decrease on myristic (P = 0.006) and palmitic (P = 0.043) for CHI animals, then PRO group, with no effect (P > 0.05) for other caecotroph fatty acids evaluated (Table 3).

Table3. Effects of probiotic and chitosan on caecotroph fatty acid profile of New Zealand white rabbits

Fatty acids (g/100g)	Chain length	Dieta[A]			SEM	*P-Value*[B]	
		CT	PRO	QUI		C1	C2
Myristic	C 14:0	2.08	2.10	2.06	0.005	0.927	0.006
Palmitic	C 16:0	26.50	26.51	26.57	0.020	0.411	0.043
Palmitoleic	C 16:1	2.38	2.39	2.37	0.005	0.991	0.368
Stearic	C 18:0	11.02	11.04	11.06	0.011	0.243	0.354
Oleic	C 18:1	25.53	25.61	25.64	0.018	0.018	0.423
Linoleic	C 18:2	29.55	29.65	29.67	0.017	0.013	0.390
Linolenic	C 18:3	0.939	0.939	0.947	0.006	0.782	0.682
Arachidonic	C 20:0	0.174	0.175	0.175	0.001	0.869	0.946
Eicosanoic	C 20:1	0.235	0.238	0.231	0.001	0.767	0.785
Behenic	C 22:0	0.242	0.242	0.243	0.001	0.966	0.356
Erucic	C 22:1	0.780	0.768	0.789	0.006	0.898	0.189
Σ saturated	-	40.02	40.07	40.12	0.024	0.233	0.273
Σ unsaturated	-	59.42	59.59	59.65	0.030	0.003	0.221
Σ monounsaturated	-	28.94	29.00	29.04	0.017	0.013	0.357
Σ polyunsaturated	-	30.48	30.58	30.61	0.017	0.006	0.656
UFA/SFA	-	1.48	1.48	1.48	0.001	0.547	0.869
Total	-	99.55	99.56	99.59	0.028	0.652	0.987

[A]Diets: CT, control, with no additives inclusion; PRO, diet with 4 g/kg of DM of probiotic inclusion; CHI, diet with 4 g/kg of DM of chitosan inclusion
[B]C1, contrast between CT and Additives; C2, contrast between PRO and CHI.

Rabbits fed with additives showed a tendency (P = 0.09) for higher values of meat b*, greater (P = 0.007) meat luminosity and an increase of 14% (P = 0.02) in CL compared to the CT group (Table 3). In addition, redness was affected by the additives, in which lower values for CHI treatment compared to PRO were detected. When analyzing the data of ΔE, it was possible to verify that the color difference was greater in the meat of the rabbits that ingested the additives, in relation to TC (P=0.012). In addition, the CHI animals had a decrease (P = 0.01) in the CL by 10.2% and a tendency (P = 0.07) for lower WBSF. In addition, there were no effects (P> 0.05), between treatments, for the meat's water retention capacity and pH (Table 3). According proximate analysis, there was no effect of additives compared to the CT group (contrast 1); however, CHI decrease the meat crude protein by

23.3% and the ether extract by 13.8% compared to PRO group (Table 4).

Table 4. Effects of probiotic and chitosan on meat quality and composition of New Zealand white rabbits

Item[A]	Diet[C]			SEM	*P-Value*[D]	
	CT	PRO	CHI		C1	C2
Color[B]						
a*	1.43	1.38	0.61	0.19	0.370	0.052
b*	1.60	2.93	2.03	0.25	0.090	0.264
L*	49.30	53.30	54.10	0.89	0.007	0.730
ΔE	49.35	53.40	54.14	0.92	0.012	0.547
h*	0.84	1.13	1.28	0.01	0.014	0.751
C*	1.99	1.51	0.61	0.02	0.031	0.124
WHC (%)	75.3	75.1	68.1	2.00	0.230	0.250
CL (%)	22.8	27.4	24.6	0.60	0.020	0.010
WBSF (kg)	1.70	2.57	1.70	0.17	0.190	0.070
pH	6.62	6.77	6.66	0.42	0.270	0.190
Proximate analyses (% of DM)						
Dry matter	31.0	28.2	27.7	1.19	0.400	0.253
Mineral matter	4.73	4.63	4.92	0.08	0.790	0.230
Crude protein	13.2	15.0	11.5	0.75	0.970	0.010
Fat	7.40	8.65	7.45	0.26	0.300	0.030

[A]WHC, water holding capacity; CL, cooking loss; WBSF, shear force.
[B]The color analysis was performed after 24 h postmortem. L* = Lightness; a* = redness; b*=yellowness; ΔE = color difference.
[C]Diets: CT, control, with no additives inclusion; PRO, diet with 4 g/kg of DM of probiotic inclusion; CHI, diet with 4 g/kg of DM of chitosan inclusion
[D]C1, contrast between CT and Additives; C2, contrast between PRO and CHI.

Animals fed additives had a 17% decreased on arachidonic acid (P = 0.022), a 2% and 6.6% increased on behenic acid (P = 0.032) and erucic acid (P = 0.025), respectively, compared to the CT group (Table 4). There was a decreased (P = 0.045) on linoleic acid on meat from animals fed CHI compared to PRO diet, as consequence the Omega-6 (P = 0.045) and USF/SFA (P = 0.071) ratio were also decreased for CHI animals compared to the PRO group. Also, CHI increased erucic acid (P = 0.017) and decreased the hypocholesterolemia index (P = 0.031) compared to rabbits fed PRO (Table 5).

Table 5. Effects of probiotic and chitosan on meat fatty acid profile of New Zealand white rabbits.

Fatty acids (g/100g)	Chain length	Diet[A]			SEM	*P-Value*[B]	
		CT	PRO	QUI		C1	C2
Myristic	C 14:0	2.07	2.06	2.07	0.008	0.804	0.767
Palmitic	C 16:0	25.9	25.8	25.8	0.012	0.112	0.477
Palmitoleic	C 16:1	2.48	2.51	2.46	0.010	0.701	0.166
Stearic	C 18:0	11.2	11.1	11.2	0.014	0.711	0.143
Oleic	C 18:1	25.8	25.8	25.8	0.017	0.979	0.456
Linoleic	C 18:2	30.1	30.2	30.1	0.013	0.233	0.045
Linolenic	C 18:3	0.89	0.90	0.89	0.005	0.434	0.451
Arachidonic	C 20:0	0.17	0.10	0.18	0.002	0.022	0.636
Eicosanoic	C 20:1	0.24	0.24	0.23	0.001	0.661	0.453
Behenic	C 22:0	0.24	0.24	0.25	0.002	0.032	0.356
Erucic	C 22:1	0.75	0.75	0.85	0.003	0.025	0.017
Σ saturated	-	39.6	39.5	39.6	0.018	0.640	0.255
Σ unsaturated	-	60.3	60.4	60.3	0.021	0.348	0.031
Σ monounsaturated	-	29.3	29.3	29.3	0.015	0.835	0.661
Σ polyunsaturated	-	31.0	31.1	31.0	0.014	0.185	0.029
Total	-	98.5	98.5	98.5	0.001	0.062	0.712
Omega-3	-	0.89	0.90	0.89	0.005	0.434	0.451
Omega-6	-	30.1	30.2	30.1	0.013	0.233	0.045
Omega-9	-	26.8	26.8	26.8	0.017	0.994	0.520
UFA/SFA	-	1.12	1.13	1.12	0.001	0.178	0.071
Omega-3/Omega-6	-	48.4	47.7	48.0	0.298	0.488	0.542
Hipercolesterolemics	-	28.0	27.9	27.9	0.015	0.163	0.660
Hipocolesterolemics	-	60.3	60.4	60.3	0.020	0.348	0.031
Hiper/Hipo	-	0.66	0.66	0.66	0.001	0.423	0.437
Thrombogenicity index	-	0.69	0.69	0.69	0.002	0.500	0.487

[A]Diets: CT, control, with no additives inclusion; PRO, diet with 4 g/kg of DM of probiotic inclusion; CHI, diet with 4 g/kg of DM of chitosan inclusion
[B]C1, contrast between CT and Additives; C2, contrast between PRO and CHI.

DISCUSSION

Over the past few decades, pharmaceutical technologies, such as antibiotics, have been used in livestock production systems to improve animal performance and reduce production costs. The most consistent effect of antibiotics is the reduction of diseases, by improve the protection against bacterial disease and, consequently, stimulate growth rates. However, with the high consumer demand for antibiotic-free animal products, some alternative additives have been studied in animal production.

According to Yamani et al. (1992), that used a complex probiotic similar to the present study (*Lactobacillus acidophilus, Streptococcus faecium*, and yeasts) for rabbits, crude fiber digestibility improved at 8 and 12 weeks age, and it lead to the greater performance of rabbits fed PRO. Bhatt et

al. (2017) also researching probiotic for growing Chinchilla rabbits, reported a greater feed conversion, average daily gain and final body weight of animals fed with *Lactobacillus acidophilus* and *Lactococcus lactis*. In addition, Oso et al. (2013) that worked with *Prediococcus acidilacts* and *Bacillus cereus*, reported improvements in the weight gains, feed conversion ratios, but not affected apparent nutrient digestibility values. However, these authors observed highest caecal lactobacillus with lowest coliform counts.

According to chitosan supplementation, Araújo et al. (2015) observed increase on rumen propionate (7%) and reduction in acetate (2%) proportion in steers fed chitosan. The authors explain that it can be associated with changes in the carbohydrate digestion without altering dry matter intake when chitosan was added in the rumen. Because of the effect of additives in performance traits, differences were also expected for serum metabolites by additives supplementation. Glucose concentration was reduced by CHI treatment compared to PRO animals, which could also represent an increase in muscle energy demand for growth in CHI-supplemented animals. Despite no statistical differences, animals fed PRO had lower ADG by 3% compared to CHI group, suggesting greater energy demand for CHI animals to growth. The cholesterol was also affected by type of additives, in which animals fed PRO had a lower value than CHI supplemented animals. According to Pereira & Gibson (2002) the probiotics have been related by their cholesterol-lowering effects and despite no sufficiently address the mechanisms by which probiotics act for this effect, several mechanisms have been hypothesized, which include enzymatic deconjugation of bile acids by bile-salt hydrolase of probiotics, assimilation of cholesterol by probiotics, cholesterol binding to cell walls of probiotics, incorporation of cholesterol into the cellular membranes of probiotics during growth, and production of short-chain fatty acids upon fermentation by probiotics in the presence of prebiotics (Ooi & Liong, 2010).

As explained by Choi et al. (2012) and complemented by Anandan et al. (2013), in the stomach of non-ruminants chitosan acts as a cationic polysaccharide due to the acidic environment, causing the positive amino groups of the fiber to bind to negatively charged molecules, such as fatty acids. Although neutrally charged triglycerides are not affected, there is a reduction in plasma LDL levels (Santas et al., 2012). This gastric content, when it reaches the intestine and forms a complex composed of fatty acids, chitosan and bile acids in a higher pH environment, undergoes precipitation (Zhang et al., 2012). After precipitation, the bound fatty and bile acids are inaccessible to enzymes (Hossain et al., 2007), followed by the digestive tract and excreted in the faeces (Anandan et al., 2013).

For these reasons, in the present study the best fatty acid profile was presented by the animals that ingested chitosan and serum triacylglycerol concentration was increased in animals fed additives, and also was greater for animals fed PRO than CHI. The transport of triacylglycerols to target tissues is made via chylomicrons that, when reaching the tissue, are broken down by the lipoprotein lipase enzyme into glycerol and fatty acids to be absorbed and oxidized by the cells (muscles; (Nelson & Cox, 2008). The increase in serum triglycerides in fed additives animals and in probiotic animals could indicate less necessity of fatty acids oxidation by greater energy metabolism, including greater propionate production for

animals fed additive (Falcão-e-Cunha et al., 2007). Which could also explain the increase on meat protein and fat accumulation in animals fed with PRO compared to CHI in the present study by proximate analyses.

Regarding protein metabolites, the lower urea concentration on animal serum supplemented with additives may reflect a decrease of protein catabolism in skeletal muscle (Van Bibber-Krueger et al. 2015) or an increase in tissue nitrogen (N) deposition (Brake et al., 2011). It can also be observed by greater average daily gain and final body weight for animals fed additives than control group. According to Falcão-e-Cunha et al. (2007) the use of probiotics in rabbits improve the protein metabolism by decreasing ammonia production, increasing liver protein synthesis, and decreasing protein losses.

The color of the meat is the main attractive factor for the consumer at the time of purchase. The present data indicated that the red color was lower in the animals with CHI than in the PRO, in addition, the additives increased the yellowing and the luminosity of the meat in comparison to the control group. Meng et al. (2010) reported that meat color scores and redness values increased when pigs received the probiotics *Bacillus subtilis* endospore and *Clostridium butyricum* endospore complex. Along the same lines, Pelicano et al. (2003) also observed that the redness values in the meat of broilers increased in the groups treated with probiotics compared to the control group.

In measuring meat quality, water retention capacity, including drip loss and cooking loss, are crucial as meat quality determination, since some nutrients are easily lost during water loss exudation processes (Chen et al., 2012). In the present study, no effect of treatments were observed for WHC between treatments; however, the additives increased the CL and PRO had also greater CL and WBSF than CHI group. Contrary to the present data, Bai et al. (2017) reported lower CL and WBSF for broiler chicken fed PRO compared to the control diet. Park & Kim (2014) concluded that dietary supplementation with probiotics (*B. subtilis*) in broiler diets increased the drip loss of breast meat after storage for one day, although the detailed reasons were unclear. Shear force was often expressed as the capacity for tenderness, and was one of the crucial sensory qualities that influenced the consumer. Zhou et al. (2010) reported that dietary *B. coagulans* exerted positive effects on the shear force of chicken breast meat. Pelicano et al. (2003) note that dietary probiotics in broiler diets was beneficial in meat quality by improving the pH, tenderness, and color. However, the incongruities in results were due to the strains of probiotics, administration dosage, methods of preparation, bird age, diet composition, and hygiene status.

The fatty acid profile of the diet offered to rabbits is mainly composed of unsaturated fatty acids and the caecotroph fatty acids profile in indicate that additives improved the C 18:1 and C 18:2 on rabbits excretes, improving as well as the Σ unsaturated, Σ monounsaturated and Σ polyunsaturated. It can be due to the microorganisms evaluated were able to changes and synthetize UFA. *Lactobacilli strains* have complex mechanisms by which different fatty acids are converted into shorter, longer, more saturated, or unsaturated fatty acids (Ross et al., 2012). There is evidence that low levels of oleic acid (18:1) in culture medium resulted in more lactobacillic acid and high levels resulted in higher amounts of dihydrosterculic acid.

Standard rabbit meat is already a quite good source of unsaturated fatty acids (UFA) and linoleic acid, in the present study, it represents about 60% and 30% of total FAs, respectively, as consequence, rabbit meat can be effectively used to produce functional meat and meat products. Generally, the use of additives affects just a few FA on meat compared to the control group, increasing C 22:0, C 20:1, C 20:0, and the total FA, it can be probably because changes on fatty acid metabolism by additives supplementation as described above.

In addition, the PRO diet increased the C 18:2, Σ unsaturated, Omega-6, UFA:SFA, and hypocholesterolemic index in meat. It can be due to two mean reasons, first because the composition of caecotroph, and second, because to the microorganisms evaluated were able to changes and synthetize UFA, as described above. This result indicate that probiotic can improve the meat healthily. In addition, the SFA:UFA ratio in the probiotic group meat was relatively close to the recommended ratio (Wood et al., 1999). Ross et al. (2012) fed pigs with probiotics, reported a decrease on C 14:0, SFA, and an improvement on C 18:3, CLA cis9-trans-11, MUFA and PUFA. The authors also explain that microorganisms evaluated were able to conjugate LA, increasing CLA, and PUFA.

CONCLUSION

The data showed that the probiotic treatment led a better meat quality, according fatty acids profile and hypocholesterolemic index compared to chitosan treatment. The additives improved the caecotroph fatty acid profile.

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