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ANIMAL SCIENCE

Intake, digestibility and ruminal parameters of lambs fed with increasing levels of wheat bulgur

FRANCISCO ANTONIO PIRAN FILHO, EMILYN MIDORI MAEDA, ANA CAROLINA FLUCK, MARIANNE CRISTINA GONÇALVES HASSE, DIANA GILIOLI, EMANOELE CRISTINA WEISS & OLMAR ANTÔNIO DENARDIN COSTA

Abstract: The aim was to evaluate the effect of the inclusion of wheat bulgur in the diet on intake, digestibility, N balance and ruminal parameters in cannulated lambs. Four castrated Santa Ines×Dorper lambs, cannulated in the rumen, (45 ± 9 kg) were housed in metabolism crates. They were randomly distributed in a 4×4 Latin Square,10-day adaption, a 6-day sampling period. The base diet was composed by ryegrass hay and concentrate, in a 40:60 roughage:concentrate ratio and four inclusions of wheat bulgur on the total diet: 0, 190, 380 and 570 g/kg dry matter. The inclusion of wheat bulgur did not affect the intake of non-structural carbohydrates. The intake of dry matter, fiber, crude protein, organic matter and crude fat decreased linearly (P<0.05). The digestibility of neutral detergent fiber decreased linearly (P=0.001). The N retained and the excreted in urine were not affected by wheat bulgur inclusion. Fecal excretion, total excretion and absorbed N, decreased linearly (P<0.05). There was no change on pH, ammonia, soluble carbohydrates and protozoa population in rumen. Increasing of wheat bulgur in the diet of lambs decrease the nutrient intake and fiber digestibility without affecting the digestibility of other compounds, ruminal parameters, and the protozoa count.

Key words: alternative feeding, ruminant, nutritional quality, ruminal biology.

INTRODUCTION

In animal production systems, feeding represents the largest portion of the cost, therefore, searching for alternative sources of food with good nutritional quality, affordability and availability is important for the success of this productive activity. The use of by-products from agricultural industries can become an alternative for formulating diets potentially reducing feeding costs (Cruz et al. 2011).

The wheat bulgur is a by-product obtained through wheat classification, consisting of well formed grain in a smaller size, composed by wheat grains that leak through oblong mesh sieves of 1.75 mm x 20.00 mm. In certain conditions, it may contain varying amounts of broken grains, hollow grains, wheat particles (husk, stems and leaf fragments) and non-wheat originated particles (dirt, stones, seeds and other plant debris) that also leak through sieves, such factors make the product unfit for human consumption. Certain wheat batches that do not reach the specific weight required for milling or meet the classification for manufacturing flour are also called wheat bulgur (Barbosa et al. 1990).

The use of wheat bulgur in the feeding of non-ruminants has already been evaluated in several studies, for feeding poultry (Nunes et al. 2001), swines (Barbosa et al. 1990, Hauschild et al. 2004) and fish (Signor et al. 2007); however, in ruminant feeding there is a lack of studies.

Some studies have evaluated wheat bulgur with other ingredients regarding the partition of carbohydrate, protein, kinetic parameters of ruminal degradation *in vitro* and nutritional value (Pegoraro et al. 2017), but no study evaluates wheat bulgur directly related to the feeding of ruminants.

The lack of information about the nutritional composition of by-products, also called alternative by-products, leads to broader or generalized recommendations of use. Therefore, the aim was to evaluate the effect of increasing levels of wheat bulgur on intake, digestibility, N balance and ruminal parameters of lambs.

MATERIALS AND METHODS

Location, animals, housing and experimental design

The experiment was carried out from December, 2016 to February, 2017 in the experimental farm of the Universidade Tecnológica Federal do Paraná (UTFPR), Dois Vizinhos *Campus*, Paraná State - Brazil, according to the norms of the Committee on Animal Research and Experimentation (protocol nº 2016-020-UTFPR).

Four Santa Ines x Dorper crossbred lambs (45±9 kg initial body weight), with rumen cannulas, were randomly housed in 0.96 m^2 metabolism crates with individual feed and water dispensers. They were distributed in a 4x4 Latin Square design to evaluate the effect of wheat bulgur dietary inclusion.

Experimental diets

Chemical analysis were carried out in order to formulate the experimental diets (Table I).

The treatments were: 0, 190, 380 and 570 g/kg of wheat bulgur in the diet (based on dry matter - DM) (Table II).

The diets were formulated for lambs with approximately 40 kg body weight and 0.250 kg average daily gain. The experimental diets were isonitrogenous and isocaloric and, formulated to meet the recommended level of crude protein (CP) and metabolizable energy (ME) in DM of 125

Table I. Chemical composition of the corn grain (CG), wheat-bulgur, ryegrass hay (RH) and soybean meal (SM) used in the experimental diets.

Item (g/kg DM)	CG	Wheat-bulgur	RH	SM
Dry matter	898.3	890.9	898.4	873.7
Organic matter	984.1	962.1	936.6	933.6
Crude protein	97.5	169.1	62.0	487.7
Crude fat	51.1	17.8	18.6	14.3
Ash	15.9	37.9	63.4	66.4
aNDFom[a]	162.0	208.0	684.4	165.2
ADFom	38.9	72.3	439.8	104.1
Lignin(sa)	9.8	20.8	64.9	10.4
NDICP	9.2	21.7	15.4	42.5
ADICP	2.7	2.5	5.4	18.4
Non-structural carbohydrates	682.7	588.9	187.0	308.9
Total carbohydrates	835.5	775.2	856.0	431.6
Total digestible nutrients	878.0	788.1	545.9	790.7

[a]aNDFom= neutral detergent fiber using a heat stable amylase and corrected for ash, without the use of sodium sulfite; ADFom= acid detergent fiber expressed exclusive of residual ash; Lignin(sa)= lignin determined by solubilization of cellulose with sulphuric acid; NDICP, neutral detergent insoluble crude protein; ADICP= acid detergent insoluble crude protein.

g/kg and 660 g/kg, respectively, considering a daily intake of 1.3 kg DM (NRC 2007).

Diets were based on ryegrass hay and concentrate feed, with a roughage:concentrate ratio of 40:60. The hay was chopped into 5–10-cm pieces using a forage chopper before offering to the lambs in order to facilitate weighing, mixing and reduce particle sorting.

In vivo trial, samplings and procedures

The animals were fed *ad libitum*, with two daily meals, at 9 am and 5 pm. After a 21-day period to adapt the animals to the diet and facilities, the experiment was completed in four 16-day periods: 10-day adaption to the diets, a 5-day sampling period, and 1-day rumen fluid sampling. The feed delivered, orts and feces were weighed daily and subsampled from days 10–15 of each experimental period, as the estimate and subsampling of 24 h urine excretion of each day. On the 16-day, it was carried out the rumen fluid sampling.

The animals were weighed on the 10-day of each experimental period, before the sampling period in order to reach 10% of orts. The control of intake and orts was performed daily by

Table II. Ingredients quantity and chemical compositions of the experimental diets containing different levels of bulgur.

	Level of bulgur in diet (g/kg DM)			
Item (g/kg DM or as stated)	**0**	**190**	**380**	**570**
Ingredient (g/kg of DM)				
Corn, grain, ground, dry	483.8	328.6	173.4	18.3
Wheat-bulgur, ground, dry	-	190.0	380.0	570.0
Ryegrass, hay	400.0	400.0	400.0	400.0
Soybean, meal	108.7	73.8	39.0	4.1
Vitamin-mineral premix[a]	7.5	7.5	7.5	7.5
Chemical composition				
Dry matter (g/kg)	886.5	884.4	885.5	885.2
Organic matter	950.8	950.5	950.6	950.3
Ash	49.2	49.5	49.4	49.7
Crude protein	136.9	136.5	133.5	130.3
Crude fat	20.1	18.3	17.2	16.0
aNDFom[b]	386.4	390.6	389.3	393.9
ADFom	204	205.9	207.7	208
Lignin(sa)	31.8	31.9	31.5	32.0
Total carbohydrates	793.9	795.7	799.9	804
Non-structural carbohydrates	464.1	458.7	464.5	465.7
Total digestible nutrients	711.1	706.7	706.5	702.6
Digestible energy (MJ/kg DM)	13.2	13.1	13.1	13.0
NDICP	56.6	53.6	53.9	55.5
ADICP	14.2	13.4	13.5	13.9

[a]Premix contained (per kg): Ca, 136 g; Na, 136g; P, 80g; Mg, 10 g; Fe, 1300 mg; Mn, 1000 mg; F, 800 mg; K, 140 mg; I, 100 mg; Co= 85mg; S= 12 mg; Zn= 2,500 ppm.
[b]aNDFom= neutral detergent fiber using a heat stable amylase and corrected for ash, without the use of sodium sulfite; ADFom= acid detergent fiber expressed exclusive of residual ash; Lignin(sa)= lignin determined by solubilization of cellulose with sulphuric acid; NDICP= neutral detergent insoluble crude protein; ADICP= acid detergent insoluble crude protein.

using the average intake obtained during the adjustment period.

In order to estimate the DM, neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude protein (CP) intake, and digestibility, total daily feces were collected twice a day, after each feeding, using collection bags attached to the animals, and removing a subsample of approximately 10% from the total amount. They were homogenized and stored in freezer at -18° C, and formed the composite sample at the end of the period. For total urine daily sampling, we used buckets containing 100 mL of 3.6 M sulphuric acid (H_2SO_4) to avoid any possible fermentation and nitrogen compounds volatilization. A 2% of total daily urine was frozen to estimate the nitrogen balance (NB) or retained nitrogen (N) through the difference between N intake and N excretion in feces and urine.

Rumen fermentation parameters and protozoa count

Rumen fluid samplings were performed on the 16th day of each experimental period, at 0, 1, 2, 3, 4, 6 and 8 h after the morning feeding. The samples were filtered and pH was immediately measured in a digital pH meter (MPA-210). Rumen fluid samples (100 ml) were acidified with 3.6 M H_2SO_4. These samples were analyzed by colorimetric methods in spectrophotometer (Biospectro SP-220) to estimate the ammonia content by the phenol-hypochlorite reaction method (Weatherburn 1967) and soluble carbohydrate content by phenol-sulfuric method (Dubois et al. 1956).

The protozoa count was proceeded with 20 ml of ruminal content. It was collected without filtering, homogenized and stored in plastic containers with 20 ml of 18.5% formalin for fixation. The count of ciliate protozoa was performed in a Sedgewick-Rafter chamber, according to Dehority (2003). Thus, 1 ml of ruminal fluid and 9 ml of glycerin at 30% were inserted and the Lugol solution was applied (100 ml of distilled water, 5 g of iodine and 10 g of potassium iodide) replacing brilliant green (D'Agosto and Carneiro, 1999). The averages of the counts made in 100 independent fields were considered, performed in duplicate and analyzed in optic microscope (K112L KASVI) with a 100x magnification.

Chemical analyses

All samples were dried in a forced air oven at 55 °C for, at least, 72 h and ground in Wiley-type mill fitted with a 1-mm-sieve. Samples of feed, orts, feces and urine were pooled on a 5-day basis within each experimental period. Chemical analysis included DM determined by oven-drying at 105 °C for 8 h (Method 967.03; AOAC, 1998), ash was estimated by placing on muffle furnace at 600 °C for 4 hours (Method 942.05; AOAC, 1998). Organic matter content (OM) was calculate as OM = 1000 – ash (g/kg). CP was estimated by the Kjeldahl method (Method 984.13; AOAC, 2006). Neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP) were determined according to Licitra et al. (1996). The analyses of NDF and ADF followed the modified methodology by Van Soest et al. (1991), using 16 μm polyester bags (Komarek 1993) and autoclave at 110°C for 40 min (Senger et al. 2008). Only for the NDF analysis there was addition of a standardized solution of heat-stable amylase (Mertens 2002). To determine lignin concentration, the residue from ADF was treated with 72% sulfuric acid (Method 973.18; AOAC, 1998). Crude fat (CF) content was determined through the fat-extractor (ANKOM^{XT15} Extraction System®, ANKOM Technology Corporation, Fairport, NY, EUA) with petroleum ether. Total carbohydrates (TC – g/kg of DM) through the equation TC= 1000– (CP+CF+Ash), and their contents were calculated according to Sniffen et al. (1992).

Statistical analysis

The data were analyzed by the model:

$$Y_{ijk} = \mu + A_i + P_j + T_k + E_{ijk},$$

In which: Yijjk = response variable, μ = mean of observations, A_i = random effect of animals, P_j= random effect of periods, T_k = fixed effect of treatments and E_{ijk} = random error. The normality was tested for each response variable, and, when necessary, the *Box Cox* data transformation was applied. The data obtained were analyzed through the *Mixed* procedure (SAS 2013). Both linear and quadratic regressions were carried out according to the levels of wheat bulgur in the diet (P=0.05). The ruminal parameters (pH, $N\text{-}NH_3$ and soluble sugars) were subjected to the command "repeated" of the *Mixed* procedure, suitable for repeated measures over time. We used the restricted maximum likelihood method (REML) for choosing the variance and covariance matrix that best fit the data, using the smallest corrected Akaike value (AICc) (Littell et al. 2006). The matrices were tested as variance component (VC), unstructured (UN) and first order autoregressive model (AR (1)). The total number of ciliate protozoa were estimated through Poisson distribution with a logarithmic connection function, by *Genmod* procedure (SAS 2013). Was used the SAS University Edition.

RESULTS

The inclusion of wheat bulgur in the diet of the cannulated lambs did not affect the intake of non-structural carbohydrates (Table III). However, the intake of DM, OM, CP, NDF and CF decreased linearly ($P < 0.05$).

The digestibility of DM, OM, CP, CF and NSC were not affected ($P > 0.05$) by the dietary inclusion of wheat bulgur (Table IV), however, the NDF digestibility decreased linearly ($P = 0.001$).

The N excreted in urine ($P = 0.262$) and N retained ($P = 0.344$) were not affected by the dietary inclusion of wheat bulgur (Table V).

However, N intake, fecal excretion, total excretion and absorbed N, decreased linearly ($P < 0.05$). The dietary inclusion of wheat bulgur had no effect on the ruminal parameters (pH, ammonia and total carbohydrates content) and the population of ciliate protozoa (Table VI).

Table III. Dry matter and chemical compounds consumed by rumen-cannulated lambs fed with diets containing different levels of bulgur.

	Level of bulgur in diet (g/kg DM)				SEM[a]	P-value	
Item (g/d)	0	190	380	570		Linear	Quadratic
Dry matter	1315	1266	1204	959	59.49	0.028	0.360
Organic matter	1250	1203	1146	912	56.50	0.028	0.359
Crude protein	188	182	174	135	8.34	0.019	0.252
aNDFom[b]	472	449	410	324	26.36	0.034	0.515
Crude fat	27.4	23.8	21.5	16.1	1.37	0.001	0.620
Non-structural carbohydrates	641	620	611	497	26.00	0.054	0.339

[a]SEM= standard error of the means. [b]aNDFom= neutral detergent fiber using a heat stable amylase and corrected for ash, without the use of sodium sulfite.

Table IV. Apparent digestibility of nutrients in diets of rumen-cannulated lambs containing different levels of bulgur.

	Level of bulgur in diet (g/kg DM)				SEM[a]	P-value	
Apparent digestibility (g/kg)	**0**	**190**	**380**	**570**		**Linear**	**Quadratic**
Dry matter	738	730	718	719	5.81	0.188	0.701
Organic matter	749	744	734	738	5.33	0.386	0.650
Crude protein	706	716	711	737	10.39	0.354	0.712
aNDFom[b]	527	493	424	427	17.44	0.001	0.400
Crude fat	775	773	763	736	12.12	0.238	0.600
Non-structural carbohydrates	938	944	946	950	2.25	0.073	0.889

[a]SEM= standard error of the means. [b]aNDFom= neutral detergent fiber using a heat stable amylase and corrected for ash, without the use of sodium sulfite.

Table V. N balance in rumen-cannulated lambs fed diets with different levels of bulgur.

	Level of bulgur in diet (g/kg DM)				SEM[a]	P-value	
Item (g/d or as stated)	**0**	**190**	**380**	**570**		**Linear**	**Quadratic**
N intake	30.05	29.18	27.79	21.59	1.33	0.019	0.252
N voided							
Faeces	8.83	8.32	8.03	5.76	0.50	0.029	0.335
Urine	15.04	12.94	13.90	12.38	0.68	0.262	0.836
Total	23.87	21.26	21.93	18.14	0.90	0.034	0.723
N absorbed	21.21	20.85	19.76	15.83	0.93	0.033	0.291
N retained							
g/d	6.18	7.91	5.86	3.45	0.87	0.344	0.312
Intake (%)[b]	19.64	25.92	20.44	16.30	2.52	0.511	0.329
Absorbed (%)[b]	28.14	36.46	28.97	21.75	3.58	0.424	0.300

[a]SEM= standard error of the means. [b]% in relation to the N intaked.

DISCUSSION

Feed intake

The use of diets that present high ruminal degradability of starch can affect negatively the DM intake (Allen et al. 2009, Vieira 2011). The increase in the supply of wheat bulgur can increase the pool of degraded carbohydrates in the rumen, causing a decrease on DM intake (Li et al. 2012). Still, the location where starch is digested affects intake (Vieira, 2011). The starch on wheat is characterized by higher digestion rate in the rumen than corn. Thus, even though they may have a lower content of non-fibrous carbohydrates, they are more soluble. With than, may can be high production of propionate (NRC 2001, Lechartier & Peyraud 2010, Moate et al. 2017). Thus, Allen et al. (2009) suggest that the elevation on propionate levels is able to decrease the intake, because it presents higher hypophagic effect than glucose or lactate, decreasing the intake by the Hepatic Oxidation. Other important fact is the reduction in the readily digestible fiber with decrease in the use of soybean meal. This factor that influenced the feed intake was the effect of rumen filling, due to the effect of reduction in fiber digestibility (Allen & Mertens 1988).

The decrease in CF intake is related to the dry matter intake. Still, the wheat bulgur showed

Table VI. **Dietary replacement of corn with different levels of bulgur on fermentation parameters in the rumen of rumen-cannulated lambs.**

	Level of bulgur in diet (g/kg DM)				SEM[a]	P-value	
Item	**0**	**190**	**380**	**570**		**Linear**	**Quadratic**
pH[b]	6.84	6.78	6.64	6.63	0.05	0.201	0.785
Ammonia-N (mg/dL)[b]	11.34	9.26	10.75	11.48	0.49	0.663	0.154
Soluble carbohydrates (mg/dL)[b]	112.96	116.27	110.86	77.08	7.10	0.065	0.170
Protozoa ($\times10^4$/mL of rumen digesta)							
Hour 0 (after feeding)	19.48	16.76	19.84	19.20	0.53	0.650	0.354
Hour 4 (after feeding)	17.08	17.68	17.80	19.12	0.38	0.064	0.623
Hour 8 (after feeding)	16.96	19.96	22.70	21.20	1.12	0.123	0.298

[a]SEM= standard error of the means. [b]Values are averages of repeated sampling of rumen fluid collected from 4 animals assigned to each treatment just before lambs were offered the morning feeding (0 h) and 2 h, 4 h, 6 h and 8 h after feeding.

lower CF content compared to the ground corn (1.78% and 5.11%, respectively), This results in lower concentrations of CF in the diet, with the increase of the by-product.

Diets with up to 19% of wheat bulgur meet the CP recommendations proposed by the NRC (2007). However, the treatment with 38% and 57% of wheat bulgur did not meet the CP requirements in the diet (135 g/kg DM), and under these conditions, the animal performance might have been compromised.

Apparent digestibility and N balance

The decrease in digestibility of aNDF with increased levels of wheat bulgur in the diet is consistent with the results found by Tripathi et al. (2007) and Leddin et al. (2009), who found that the inclusion of wheat in ruminant diet promoted reduction in aNDF digestibility. The decrease in digestibility of aNDF according to increased levels of wheat bulgur is due to the fact that the wheat bulgur has higher amout of structural carbohydrates with slow or incomplete digestion compared to corn and soybean meal (20.8, 9.8 and 10.4 g/kg of lignin, respectively). Liu et al. (2016) verified that the inclusion of increasing levels of wheat until complete replacement of corn, for supplementation of beef cattle, resulted in reduced digestibility in aNDF and ADF. However, there was no influence on the DM, OM and CP apparent digestibility with the inclusion of wheat bulgur. Probably, the decrease in the soluble fiber showed a higher connection to the decrease on NDF intake and consequent decline in its digestibility, showing no effect on the digestibility of any other compounds.

The results on retained N and, N balance reflects the concentration of N-NH_3 no undergone any changes relationated mainly with the protein digestibility. It shows that the N excess is absorbed in the ammonia form and excreted through urine (McDonald et al. 2011).

Still, the results of protein digestibility and N fecal show relationship with the decrease in the soluble fiber quantity, that is present in the corn (Nuez Ortín and Yu, 2009) and soybean meal, with inclusion of wheat bulgur. Thus, the higher time of the digesta in the rumen contributes to the degradability of other soluble compounds. The quantity of insoluble nitrogen in the soybean meal (Table I) can contribute to this result. However, the lower intake of nutrients (Table III) with the inclusion of wheat bulgur reflected in a lower supply of N and, consequently, less quantity of absorbed N.

Rumen fermentation parameters and protozoa count

The similar values of pH observed can be explained through the quantity of roughage provided (40% of DM) for all treatments. The fiber content in the diet has a positive relationship with pH, as it stimulates chewing and, consequently, saliva production (NRC 2001), this may have led to the buffering effect on the rumen pH. In addition, the absence of pH variation between treatments, with the inclusion of wheat bulgur, is related to the similar amounts of fiber and non-structural carbohydrates in the diet. Lechartier & Peyraud (2010) report a strongly correlation between ruminal pH and the amount of NDF in the forage. Other fact that may have contributed to the control of ruminal pH was the similar values of ammonia in the treatments.

Ruminal N-NH_3 concentrations did not varied between treatments and, they are considered suitable (5-80 mg/dL) for microbial protein synthesis (Satter & Roffler, 1975, McDonald et al. 2011). Oscillations in the amount of ammonia dissolved in rumen are directly related to the degradation rate of energy and protein fractions. If this synchronization fails, there may be an elevation of ammonia concentration in the ruminal environment. Lechartier & Peyraud (2010) found that diets with fast rumen degradability presented lower ruminal N-NH_3 concentrations when compared to diets with slower ruminal degradation. Likewise, these authors comment on the fact that the increase in ammonia concentration is related to the reduction of roughage in the diet. Thus, the results show that there was no change in the synchronization of the degradation rates of energetic and protein compounds.

The soluble carbohydrates in the ruminal environment did not varied, showing the relationship with pH. Still, there was a trend in the decrease of soluble carbohydrates concentration in rumen with the wheat bulgur increase. In excess, soluble sugars can negatively affect pH and digestibility. However, Kozloski et al. (2006) related that a low concentration of sugars in rumen can reduce the activity of the bacteria due to the low energy availability. This fact may be related with the NDF digestibility in our study. Thus, the decrease in soybean meal and ground corn may have contributed to the decrease in soluble carbohydrates in rumen, given the decrease in the soluble fiber of these ingredients. As previously mentioned, in addition to influence the digestible fraction of NDF, with the inclusion of wheat bulgur, there was an increase in insoluble fractions of the fiber (Table I), it can also have an effect on the content of soluble carbohydrates in the rumen.

The count of ciliate protozoa did not show any change. The similar pH values between the treatments can be the explanation, because the reduction in pH causes decreases in the total number of rumen protozoa (Dayani et al. 2007, Moate et al. 2017).

CONCLUSIONS

Increasing levels of wheat bulgur in the diet of lambs decrease the nutrient intake and fiber digestibility without affecting the digestibility of other compounds, ruminal parameters, and the protozoa count.

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FRANCISCO ANTONIO PIRAN FILHO[1]
https://orcid.org/0000-0002-9324-7584

EMILYN MIDORI MAEDA[2,3]
https://orcid.org/0000-0001-8953-3935

ANA CAROLINA FLUCK[2,4]
https://orcid.org/0000-0001-9133-2446

MARIANNE CRISTINA GONÇALVES HASSE[2]
https://orcid.org/0000-0001-6234-3269

DIANA GILIOLI[2]
https://orcid.org/0000-0002-0340-1809

EMANOELE CRISTINA WEISS[3]
https://orcid.org/0000-0001-7831-8113

OLMAR ANTÔNIO DENARDIN COSTA[4]
https://orcid.org/0000-0001-5201-2618

[1]Universidade Estadual de Maringá, Animal Science Research Program, Av. Colombo, 5790, Jd. Universitário, 87020-900 Maringá, PR, Brazil

[2]Universidade Tecnológica Federal do Paraná, Animal Science Degree Course, Dois Vizinhos Campus, Estrada para Boa Esperança, Km 4, 85660-000 Dois Vizinhos, PR, Brazil

[3]Universidade Tecnológica Federal do Paraná, Animal Science Research Program, Dois Vizinhos Campus, Estrada para Boa Esperança, Km 4, 85660-000 Dois Vizinhos, PR, Brazil

[4]Universidade Tecnológica Federal do Paraná, Postdoctoral Student of the Animal Science Research Program, Dois Vizinhos Campus, Estrada para Boa Esperança, Km 4, 85660-000 Dois Vizinhos, PR, Brazil

Correspondence to: **Ana Carolina Fluck**
E-mail: anacarolinafluck@yahoo.com.br

Author contributions

F.A.P.F. - conception and design, acquisition of data, drafting the article, and critical review of important intellectual content; E.M.M. – conception, design and critical review; A.C.F - conception and design, acquisition of data, drafting the article, and critical review of important intellectual content; M.C.G.H. – acquisition of data; D.G. – acquisition of data; E.C.W. – acquisition of data; OADC – analysis, interpretation of data and critical review of important intellectual content.