

Digestibility of canola seeds treated by autoclaving and/or condensed tannins^a

Digestibilidad de la canola tratada en autoclave y/o con taninos condensados

Digestibilidade de grãos de canola tratados em autoclave e/ou com tanino condensado

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Summary

Background: plant proteins are susceptible to rapid degradation in the rumen therefore it is important to explore the best way to improve protein utilization. **Objective:** to evaluate the effect of heat treatment and/or condensed tannins on ruminal degradability and *in vitro* digestibility of crude protein (CP) and dry matter (DM) of canola seeds. **Methods:** *in situ* and *in vitro* DM and CP digestibility of canola seeds treated with water (control; CCL), heat in autoclave (CLE), condensed tannin (CTN), and condensed tannin followed by autoclaving (CTA) were evaluated. **Results:** the DM effective degradability values (EDDM) by CCL, CLE, CTN, and CTA were 66.8%, 73.6%, 58.5%, and 77.5%, respectively. Effective degradability of crude protein (EDCP) by CCL, CLE, CTN, and CTA at a 5%/h passage rate (k) was 75.2, 77.2, 60.2, and 80.5%, respectively. Addition of condensed tannin and/or autoclaving reduced both DM and CP digestibility. **Conclusions:** treatment with condensed tannins protected canola seeds DM and CP from ruminal degradability, while treatment with heat or tannins combined with heat showed the opposite effect, increasing degradability of those fractions. Addition of condensed tannins and/or autoclaving decreased *in vitro* DM and CP digestibility.

Key words: *chemical treatment, in situ degradability, in vitro digestibility, protein source, thermal treatment.*

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Resumen

Antecedentes: es importante estudiar la mejor manera de utilizar fuentes de proteínas vegetales, ya que son rápidamente degradadas en el rumen. **Objetivo:** evaluar el efecto del tratamiento térmico y/o adición de taninos condensados sobre la degradabilidad ruminal y digestibilidad *in vitro* de la proteína bruta (PB) y la materia seca (MS) en granos de canola. **Métodos:** se evaluó la degradabilidad *in situ* y la digestibilidad *in vitro* de la MS y PB en granos de canola tratados con agua (control - CCL), térmicamente utilizando autoclave (CTE), taninos condensados (CTN), y taninos condensados seguido por autoclavado (CTA). **Resultados:** los valores de degradabilidad efectiva de la materia seca (DEMS) para CCL, CTE, CTN, y CTA fueron 66,8, 73,6, 58,5 y 77,5% respectivamente; y para la degradabilidad efectiva de la proteína bruta (DEPB) fueron 75,2, 77,2, 60,2 y 80,5%, respectivamente –a una tasa de pasaje (k) de 5%/h. La adición de taninos condensados y/o tratamiento térmico provocó la reducción de los valores de digestibilidad, tanto de la MS como de la PB. **Conclusiones:** el tratamiento con taninos condensados protegió la MS y la PB de las semillas de canola de su degradación ruminal. Los tratamientos con calor húmedo y asociación taninos más calor mostraron el efecto contrario, promoviendo el aumento de la degradabilidad ruminal de esas fracciones. La adición de taninos condensados y/o el autoclavado resultaron en menores valores de digestibilidad para la MS y PB.

Palabras clave: *degradabilidad in situ, degradabilidad in vitro, fuentes de proteína, tratamiento químico, tratamiento térmico.*

Resumo

Antecedentes: é importante estudar a melhor maneira de utilizar as fontes de proteína vegetal, uma vez que essas são normalmente degradadas no rúmen. **Objetivo:** avaliar o efeito do tratamento térmico e/ou a adição de tanino condensado sobre a degradabilidade ruminal *in situ* e a digestibilidade *in vitro* da proteína bruta (PB) e da matéria seca (MS) de grãos de canola. **Métodos:** avaliou-se neste trabalho a degradabilidade *in situ* e a digestibilidade *in vitro* da MS e PB de grãos de canola tratados com água (controle – CCL), termicamente em autoclave (CTE), tanino condensado (CTN) e tanino condensado seguido de autoclave (CTA). **Resultados:** os valores de degradabilidade efetiva da matéria seca (DEMS) para CCL, CTE, CTN e CTA foram 66,8; 73,6; 58,5 e 77,5% respectivamente, e para a degradabilidade efetiva da proteína bruta (DEPB) foram 75,2; 77,2; 60,2 e 80,5%, para taxa de passagem (k) igual a 5%/h. A adição de tanino condensado e/ou tratamento com autoclave provocaram a diminuição da digestibilidade, tanto da MS como da PB. **Conclusões:** concluiu-se que o tratamento com tanino condensado promoveu efetiva proteção da MS e PB dos grãos de canola frente à degradabilidade no rúmen. Já o tratamento com calor úmido e a associação tanino-calor, mostraram efeito contrário, promovendo aumento da degradabilidade ruminal dessas frações. A adição de tanino condensado e/ou tratamento com autoclave diminuíram a digestibilidade da MS e da PB.

Palavras chave: *degradabilidade in situ, digestibilidade in vitro, fontes protéicas, tratamento químico, tratamento térmico.*

Introduction

Environmental concerns are increasing in all production sectors. An important way to reduce environmental damages in dairy production systems is to diminish fecal production and excretion of pollutants. Presently, nitrogen excretion from milk production reaches 43.5% of the rate produced in 1944 (Carper *et al.*, 2009). This reduction is explained by the increasing and constant improvement in productive efficiency (Jerszurki *et al.*, 2010). Therefore, it is important to maximize feed nutrient utilization by the animals in order to maintain this level of efficiency.

Highly-productive dairy cows present the highest nutritional requirements during early lactation. Researchers have shown positive results when supplementing their diets with low ruminal degradability animal protein sources, such as fish and meat (Rodrigues Filho *et al.*, 2003). However, the use of these sources in ruminant feeding is currently banned. Thus, it is important to study the best way to use vegetable protein sources, since they are rapidly degraded in the rumen. Several studies using different methods of feed and diet processing have been conducted to improve protein utilization by the animals (Mjoun *et al.*, 2010; Dschaak *et al.*, 2011).

According to Waghorn (2008), condensed tannins (CT) have the ability to bind dietary protein, reducing its ruminal degradation. When protein exceeds the nutritional requirement this effect improves animal performance. However, for low crude-protein (CP) and high-fiber diets the presence of CT is often harmful.

Tannins have affinity for proteins with more hydrophobicity, indicating that hydrophobic bonds participate in the formation of complexes between proteins and tannins (Min *et al.*, 2003). Hydrophobic groups—normally oriented towards protein—are reordered to the outside after heat exposure increasing their hydrophobic surface (Lehninger, 2011) and, consequently their affinity for tannins (Min *et al.*, 2003). Tannin-protein binding is due to hydrophobic bonds and H bridges, which are pH-dependent and can dissociate under pH of the abomasum, releasing grain proteins for further intestinal digestion (Waghorn, 2008).

However, the amount of tannins in the diet should be considered, as high CT concentrations (6.51% of DM) could decrease digestion (Frutos *et al.*, 2002; Waghorn and McNabb, 2003). The objective of the present study was to estimate the effect of heat treatment and/or CT addition on ruminal degradability and *in vitro* digestibility of CP and dry matter (DM) in canola seeds.

Materials and methods

The experiment was approved by the Ethics Committee for Animal Experimentation of Universidade Estadual de Maringá (08/07/2008, number 036/2008) and conducted at Iguatemi Experimental Farm of the same university (UEM), in Paraná, Brazil.

Canola seeds were first ground using a sieveless mill. The first treatment consisted of 100 g canola seeds (DM basis) added with 100 ml distilled water and then dried in an oven with forced ventilation at 55 °C for 48 hours, as a control treatment (CCL). In the second treatment, 100 g canola seeds (DM basis) were submitted to autoclave at 127 °C (pressure: 1.5 kgf/cm²) for

30 min (CLE). The third treatment consisted of 6.48 g condensed tannin per 100 g canola seeds (DM basis) (CTN). The fourth treatment consisted of 6.48 g condensed tannin per 100 g canola seeds (DM basis), followed by thermal processing in autoclave at 127 °C (pressure: 1.5 kgf/cm²) for 30 min (CTA). After treatments, canola seeds were stored in plastic bags in a freezer at -20 °C. Ultimately, this study aimed to evaluate affects of physical and chemical treatments on canola seeds, which will be further included in concentrate feeds or in total mixed rations. Consequently CT concentration will be diluted to acceptable levels when the total diet is supplied to the animals.

Two ruminally-cannulated cows were used to evaluate *in situ* degradability and *in vitro* digestibility of DM (IVDDM) and CP (IVDCP), with five replicates per treatment. A total mixed ration (TMR) composed of 56% corn silage and 44% concentrate (approximately 24 kg corn silage and 6 kg concentrate plus mineral mixture) was offered three times a day (at 800, 1300 and 1600 h) to cows for *ad libitum* intake (Table 1). The diet was formulated to meet requirements of cows yielding 16 liters/day with 3.5% fat, according to NRC (1989). The diet was supplied to the animals for 21 days (adaptation period). Ruminal fluid was collected on day 22 for *in vitro* and *in situ* incubations.

Table 1. Composition of the diet fed to fistulated dairy cows.

Ingredients	% of total mixed diet		
Corn silage	56		
Soybean meal	10.12		
Canola meal	9.06		
Wheat meal	6.6		
Corn	17.78		
Mineral mix*	0.44		
Nutrients ¹	Concentrate	Corn silage	Total mixed diet
Dry matter (%)	89.0	27.6	54.62
Ashes (% of DM)	6.4	5.5	5.88
Crude protein (% of DM)	26.1	6.1	14.9
NDF (% of DM)	5.7	65.5	39.18
ADF (% of DM)	6.6	40.4	25.52
Ether extract	2.2	3.2	2.76

¹NDF = neutral detergent fiber, ADF = acid detergent fiber.

*Ca: 156 g/kg, P: 51 g/kg, S: 20 g/kg, Na: 93 g/kg, K: 28 g/kg, Mg: 33 g/kg, Fe: 2000 mg/kg, Cu: 400 mg/kg, Co: 30 mg/kg, Cr: 10 mg/kg, I: 40 mg/kg, Se: 15 mg/kg, Zn: 1700 mg/kg, F: maximum 510 mg/kg Mn: 1350 mg/kg, Vit. A: 135000 U.I./kg, Vit. D: 68000 U.I./kg, Vit. E: 450 mg/kg.

The DM and CP Dry degradability of canola seeds were assessed by *in situ* incubation using the nylon bag technique, according to Vanzant *et al.* (1998). Nylon bags (5 x 7 cm and 53 μ m of porosity; Ankom Technology Co., Fairport, NY, USA) were used for rumen incubation. Canola seeds were ground using a sieveless mill and placed into bags. Bags were incubated in duplicates into a stainless steel cylindrical rod (540 g) and remained submerged in the ruminal fluid. The rod was attached to the cannula by a 60 cm nylon rope. Incubation times were 0, 2, 4, 8, 12, 24, and 48 hours, and all bags were removed from the rumen at the same time (Vanzant *et al.*, 1998). All bags were washed after incubation process in a washing machine (four cycles of 10 minutes each), including the non-incubated bags (time 0). Then, all bags were dried in a forced ventilation oven at 55 °C for 72 hours.

The DM and CP degradation values were calculated by the proportion of feed that remained in the bags. However, correction for bacterial contamination was not performed. The DM and CP degradation were calculated using the equation described by Aerskov & McDonald (1979), as follows:

$$p = a + b(1 - e^{-ct})$$

Where:

“p” is degradation rate at time “t”

“a” is the DM or CP fraction that is soluble in the rumen

“b” is the DM and CP fraction that is potentially degradable

“c” is the constant rate of fraction “b” degradation

“t” is the incubation time.

Non-linear parameters “a”, “b”, and “c” were estimated by the iterative least squares procedure (SAS, 1985). Effective degradability of DM (EDDM) and CP (EDCP) in the rumen were calculated with the model of Ørskov & McDonald (1979), as follows:

$$\text{EDDM or EDCP} = a + (b \times c)/(c + k)$$

Where:

“k” is the estimated rate of solids passing into the rumen. The other parameters are similar to the described above. Effective degradation of DM and CP were estimated for each treatment, assuming a ruminal passage rate of 5%/h, assigned to the average level of intake (ARC, 1984).

In vitro digestibility of DM (IVDDM) and CP (IVDCP) were performed according to the methods described by Tilley & Terry (1963) with the modifications described by Holden (1999), using F57 bags and an artificial rumen developed by ANKOM (Ankom Technology Co., Fairport, NY, USA). The samples were analyzed for DM and CP (Kjeldahl method) according to the AOAC (1990).

The observed IVDDM and IVDCP degradation values were subjected to ANOVA using a completely randomized design in a 2 x 2 factorial arrangement with two levels of heat (absence and presence) and two levels of condensed tannins (absence and presence). Data were analyzed using the MIXED procedure of the SAS program (2003). The model was:

$$Y_{ijk} = \mu + T_i + C_j + I_{ij} + E_{ijk}$$

Where:

Y_{ijk} is the observed value of variables on grain subjected to treatment i, which received heat j and repetition k

μ is the general mean

T_i is the condensed tannins effect (absence or presence), where i = 1 and 2.

C_j is the heat effect (absence or presence), where j = 1 and 2

I_{ij} is the interaction effect of condensed tannins and heat

E_{ijk} is the random error associated with each observation.

Results

Approximately 21 g CCL /100 g DM readily disappeared from the rumen (Table 2). The potentially degradable fraction (b) was 67.80 g/100 g and the respective ruminal disappearance rate (c) was 10.54 g/100 g. The EDDM was 66.79 g/100 g for a 5%/h solid passage rate (k).

Table 2. *In situ* DM and CP degradability, effective DM and CP degradability (EDDM and EDCP, respectively), *in vitro* DM and CP digestibility (IVDDM and IVDCP, respectively) of canola seeds submitted to or withheld from heat treatment with or without condensed tannins.

Variables ¹	Treatment means					P-value ³		
	CCL	CLE	CTN	CTA	SE ²	H	T	I
DM (g/100g)								
a	20.88	22.18	14.78	27.69	5.00	0.0005	NS	0.0009
b	67.80	62.52	83.56	61.32	9.75	0.0051	0.0290	0.0196
c	10.54	23.34	5.55	22.41	8.59	0.0036	NS	NS
EDDM	66.79	73.61	58.5	77.45	7.89	0.0010	0.0176	0.0009
IVDDM	59.43	48.53	45.73	47.45	0.27	0.0001	0.0001	0.0001
CP (g/100g)								
a	31.91	26.63	10.51	26.63	8.61	0.0006	0.0001	0.0001
b	60.89	62.23	89.00	64.43	13.26	0.0039	0.0016	0.0030
c	12.31	21.70	6.09	24.66	8.56	0.0153	NS	NS
EDCP	75.18	77.20	60.18	80.53	8.48	0.0009	0.0057	0.0015
IVDCP	73.93	55.95	54.80	51.90	0.16	0.0001	0.0001	0.0001

¹a: soluble portion; b: potentially degradable portion; c: constant rate of degradation of fraction b; EDDM: effective degradability of dry matter with passage rate of 5%/h; IVDDM: *in vitro* digestibility of dry matter, IVDCP: *in vitro* digestibility of crude protein; EDCP: effective degradability of crude protein with passage rate of 5%/h; IVDCP: *in vitro* digestibility of crude protein.

²Mean standard errors (SE).

³H: heat; T: tannin; I: interaction.

Autoclaving increased ($P < 0.05$) canola's DM soluble fraction (CLE; 22.18 g/100 g) compared to the control treatment (CCL; 20.88 g/100 g) and the tannin addition (CTN - 14.78 g/100 g). Solubility of this fraction was increased (CTA; 27.69 g/100 g) when seeds were treated with CT and passed through heat treatment. As observed, tannin addition (CTN) resulted in the lowest DM solubility. However, the potentially DM degradable fraction (b) was lower when seeds were submitted to heat treatment. For the seeds that received CT, the fraction b had a significantly higher value ($P < 0.05$) compared to the other treatments, which was not expected. Nevertheless, a significant interaction was observed ($P < 0.05$) between the addition of tannins and heat treatment, promoting the reduction of fraction b, whereas tannins alone had an opposite effect.

Degradation rate of fraction b (described by the estimated parameter c) was significantly affected by heat, increasing CLE degradation rate (23.34 g/100 g) and CTA (22.41 g/100 g) compared to the control treatment (CCL; 10.54 g/100 g) and tannin addition (CTN; 5.55 g/100 g).

Canola's EDDM was increased by the heat treatment (CLE; 73.61 g/100 g of DM) and also by heat plus CT addition (CTA; 77.45 g/100 g of DM). However, EDDM had a slight reduction when only CT was added (CTN; 58.5 g/100 g of DM) compared to the control treatment. The IVDDM was higher in the control treatment, showing a positive effect of heat and tannins, as these treatments enabled an IVDDM reduction.

Regarding CP degradability, the soluble protein content (a) in canola treated with CT and/or heat ranged from 10.51 to 31.91 g/100 g of the total protein fraction. The interaction shows that heat and/or tannin addition decreased soluble protein fraction for the highest CCL (31.91 g/100 g). Moreover, tannin treatment was more effective to reduce soluble protein compared to tannins followed by heat. Fraction b for EDCP—potentially degradable portion—remained high in tannin treatments and low in the heat treatment.

The potentially degradable protein in fraction b varied from 60.89 to 89.00 g/100 g. Results were similar to those reported by Christensen & McKinnon (2004), who observed a variation from

60 to 77 g/100 g. Thus, CT addition decreased solubility of the protein fraction and increased the proportion of potentially degradable protein. The ruminal degradation rate of fraction *b* (*c*) had values between 6.09 and 24.66 g/100 g. These results were higher than those reported by Christensen & McKinnon (2004) and Dos Santos *et al.* (2004).

Canola's EDCP ranged from 60.18 to 80.53 g/100 g, and tannin addition decreased CP effective degradability (CTN; 60.18 g/100 g). However, heat treatment promoted its increase (CLE; 77.20 g/100 g). In addition, when there was a combination of both treatments (heat and tannin addition) EDCP increased significantly (CTA; 80.53 g/100 g).

The lowest IVDCP (Table 2) was observed when canola was treated with tannins and autoclaved (CTA; 51.90 g/100 g), followed by seed-autoclaving only (CLE; 54.80 g/100 g) and tannin addition (CTN; 55.95 g/100 g).

Discussion

The DM degradation rates were in agreement with those reported by Dos Santos *et al.* (2004) who observed the following values for the control treatment (without heat or tannic acid): 28.3 g/100 g degradation for fraction *a*, 65.4 g/100 g for fraction *b*, and 8.9 g/100 g for fraction *c*. Soares *et al.* (2010), working with lupin seeds and three tannin levels (0, 9.6, 18 g/100 g of protein), observed that CP soluble fraction *a* decreased significantly, while fraction *b* increased, and degradation rate *c* decreased with increasing tannin levels. Similarly in our study, when comparing CCL and CTN, tannin addition showed the same variation pattern for DM fraction *b* and CP fractions *a* and *b*.

Tannin addition associated with heat was able to reduce the potentially degradable fraction of DM in the rumen when compared to CCL and CLE, whereas CT addition was not sufficient for this purpose, moreover showing an elevated result when compared to the other treatments. These assertions are demonstrated by the high fraction *b* value in treatments merely receiving tannins, and also by the interaction between tannin addition and heat

treatment, which possibly promoted the reduction in fraction *b*.

The observed EDDM increase by heat and its reduction by condensed tannins is in agreement with Dos Santos *et al.* (2004) who also worked with heat-treated canola seeds added with tannic acid. However, the present results are opposite to those observed by Loyola *et al.* (1999) who worked with canola meal treated by autoclave and CT. These differences could be attributed to the fact that canola seeds were used in the current experiment instead of canola meal, and both differ in fiber, protein, and oil content.

The results of *in vitro* DM digestibility differ from those obtained by Dos Santos *et al.* (2004) who found no difference between canola seeds treated with heat and tannic acid compared to control.

According to Waghorn (2008), dietary CT for ruminants reduces DM digestibility as well as digestion rate. In addition, CT effects in the rumen are known, but the mechanisms that reduce the protein degradation, ammonia production, microbial growth, digestion rate, and consumption are not clear.

In summary, it is well established that tannins bind dietary protein present in saliva and microbial proteins, showing greater affinity for hydrophobic proteins. This connection is facilitated by pH around 6 and its proteolysis occurs at pH 3. It is also known that cellulolytic bacteria are more affected by the presence of tannin than cellulolytic fungi and these effects are influenced by tannin source, concentration, diet composition, and nutrient requirements of the animal (McSweeney *et al.*, 2001; Min *et al.*, 2005; Waghorn, 2008).

The CP degradability was higher for heat treatment compared with tannin addition. Similar results were reported by Christensen & McKinnon (2004). On the contrary, Dos Santos *et al.* (2004) observed protein solubility reduction when canola seeds were submitted to heat. Likewise, authors also found an interaction between heat treatment

and tannin addition. However, heat decreased and tannin increased fraction *a* of CP degradability.

The EDCP increase by heat and the combination of both heat and tannin was unexpected. In fact, further EDCP reduction would be a more logical response. Dos Santos *et al.* (2004) found no significant reduction in EDCP when tannic acid and autoclave were used separately. However, the authors observed that the association of both promoted a significant EDCP reduction.

According to several studies (Mir *et al.*, 1984; Mustafa *et al.*, 1998; Mustafa *et al.*, 2002; Wright *et al.*, 2005) appropriate that heat treatment applied to oilseeds such as soybeans, canola, peas, and flax seed reduces NH₃ ruminal synthesis without impairing intestinal digestibility, being effective in reducing ruminal protein degradability. However, the results of the present study for EDCP and EDDM are opposite to those presented by the previous authors cited, since higher effective degradability values were observed when canola seeds were submitted to heat treatment. In addition, Waghorn (2008) affirms that the tannin-protein binding is due to hydrophobic interactions and hydrogen bonds, which are pH dependent and can be dissociated in the abomasum, releasing the protein for intestinal digestion. The tannin-protein bonds depend on several factors, such as a special configuration of protein molecules and the availability of reactive phenolic groups (Loyola *et al.*, 1999). Apparently, slight differences in chemical composition of the feeds associated or not with physic-chemical treatments can result in remarkable alterations of DM and CP degradability.

Indeed, CT decreases degradation of ruminal protein through interactions between reactive hydroxyl groups and the carbonyl groups of proteins, forming indigestible complexes either by interaction of proteolytic enzymes (interfering with the protective mucous epithelium of the intestine) or by changing the absorption of digested nutrients (Loyola *et al.*, 1999; Min *et al.*, 2003; Waghorn, 2008).

According to the results found in the present study, treating canola seeds with condensed tannins

can promote an effective protection of DM and CP ruminal degradation. On the other hand, associated treatment of canola seeds with humid heat and tannins addition did not provide protection against ruminal degradation. Condensed tannin addition and/or autoclaving reduce both DM and CP digestibility.

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References

- Association of Official Agricultural Chemists (AOAC). Official Methods of Analysis. 1990. Washington. 1990.
- Agricultural Research Council – ARC. The nutrient requirements of ruminant livestock. Commonwealth Agricultural Bureaux. Farnham Royal. U.K. 1984.
- Capper JL, Cady RA, Bauman DE. The environmental impact of dairy production: 1944 compared with 2007. *J Animal Sci* 2009; 87:2160-2167.
- Christensen DA, Mckinnon JJ. Canola meal feed industry guide. Canola Council Publications.[Access date: September 2, 2004]. URL: <http://www.canolacouncil.org/pubs/cattle.html> p.1-5, 2004.
- Dos Santos WBR, Dos Santos GT, Da Silva-Kazama DC, Cecato U, De Marchi FE, Visentainer JV, Petit HV. Production performance and milk composition of grazing dairy cows fed pelleted or non-pelleted concentrates treated with or without lignosulfonate and containing ground sunflower seeds. *Anim Feed Sci Techn* 2011; 169:167-175.
- Dos Santos GT, Damasceno JC, Jobim CC, Gonçalves GD, Chiquitelli Neto M, Porto PP, Ribeiro CR. Efeito dos tratamentos com autoclave e/ou ácido tânico na degradabilidade *in situ* e na digestibilidade *in vitro* de grãos de canola. *Acta Anim Sci* 2004; 26:507-512.
- Dschaak CM, Williams MS, Holt JS, Eun AJ, Young BRM. Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation and milk production of lactating dairy cows. *J Dairy Sci* 2011; 94:25-58.
- Frutos P, Hervás G, Ramos G, Giráldez FJ, Mantecón AR. Condensed tannin content of several shrub species from a mountain area in northern Spain, and its relationship to various indicators of nutritive value. *Anim Feed Sci Techn* 2002; 95:215-226.

- Holden L. A Comparison of methods of *in vitro* matter digestibility for tem feeds. *J Dairy Sci* 1999; 82:1791-1794.
- Jerszurki D, Navarro BR, Santos GT, Almeida R. Eficiência na utilização o nitrogênio dietético em rebanhos leiteiros especializados e suas implicações ambientais. In: Reunião Anual Da Sociedade Brasileira de Zootecnia, 47, 2010. Salvador. Anais... Salvador, BA: Sociedade Brasileira de Zootecnia, 2010.
- Lehninger AL, Nelson DL, Cox MM. Princípios de Bioquímica de Lehninger. 5th Ed. São Paulo: Sarvier; 2011.
- Loyola VR, Dos Santos GT, Zeoula LM, Bett V, Pereira ALT. Degradabilidade *in situ* do farelo de canola tratado com calor e/ou tanino. *Rev Bras Zootec* 1999; 28:598-604.
- Min BR, Barry TN, Attwood GT, McNabb WC. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Anim Feed Sci Techn* 2003; 106:3-19.
- Mir Z, MacKeod GK, Buchanan-Smith JG, Grieve DG, Grovum WL. Methods for protecting soybean and canola proteins from degradation in the rumen. *Can J Anim Sci* 1984; 64: 853-865.
- McSweeney CS, Palmer B, McNeil DM, Krause DO. Microbial interactions with tannins: nutritional consequences for ruminants. *Anim Feed Sci Techn*, 2001; 91:83-93.
- Mustafa AF, Christensen DA, McKinnon JJ. Effects of moist heat treatment on crude protein composition and degradability of field peas. *Can J Anim Sci* 1998; 78:453-456.
- Mustafa AF, Gonthier C, Ouellet DR. Effects of extrusion of flaxseed on ruminal and post-ruminal nutrient digestibilities. *Arch Anim Nutr* 2002; 57:455-463.
- National Research Council – NRC. Nutrient requirements of dairy cattle. 6^a Ed. Washington DC: National Academy Press; 1989.
- Ørskov ER, McDonald I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J Agric Sci* 1979; 92:499-503.
- Rodrigues Filho M, Mancio AB, Lana RP, Cecon PR, Da Silva FF, Rodrigues NEB, Veloso CM. Desempenho e características de carcaça de novilhos de origem leiteira alimentados com diferentes níveis de concentrado e de cama de frango. *Rev Bras Zootec* 2003; 32:672-682.
- Soares BAM, Soares-Pereira ALP, Matos TJS. Preliminary results on the effects of grape (*Vitis vinifera*) seed condensed tannins on *in vitro* intestinal digestibility of the lupin (*Lupinus angustifolius*) seed protein fraction in small ruminants. *J Anim Physiol Anim Nutr* 2010; 95:456-460.
- Tilley JMA, Terry RA. A two-state technique for *in vitro* digestion of forage crops. *J Brit Classl Soc* 1963; 18:104-111.
- Vanzant ES, Cochran RC, Titgemeyer EC. Standardization of *in situ* techniques for ruminant feedstuff evaluation. *J Anim Sci* 1998; 76:2717-2729.
- Waghorn GC. Recent advances in animal in Australia. In: Proceeding of a symposium at University of New England. R. B. Cumming. Ed. Armidale, Australia. p. 24-27. 1985.
- Waghorn GC, McNabb WC. Consequences of plant phenolic compounds for productivity and health of ruminantes. *Proc Nutr Soc* 2003; 62:383-392.
- Waghorn GC. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production - Progress and challenges. *Anim Feed Sci Techn* 2008; 147:116-139.