

Tannins of tropical shrub-like legumes: their effect on protein protection of soybean meal

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For determining the potential of tannins extracted from two tropical shrub-like legumes (*Albizia lebbekoides* and *Acacia cornigera*) in the protection of soybean meal proteins and to establish their effects on fermentation indicators, the *in vitro* gas production technique was applied. A random block design was used. Untreated soybean meal (800 mg) were incubated and treated with aqueous extract of *A. lebbekoides* and heat, *A. cornigera* extract and heat, water and heat and only with heat. Treatments were incubated alone and with two levels of soluble starch (150 and 300 mg) for estimating N solubility of the substrate. Gas production was measured until 8 h and short chain fatty acid production and ammonia were determined. There was no effect of the treatments on the production of total SCFA, only the treatment with heat increased gas production ($P < 0.05$). Neither were there differences in the productions of individual SCFA nor in the acetic/propionic relationship. Albizia and acacia tannins decreased the NH_3 levels regarding the untreated substrate ($P < 0.05$), although the treatment with acacia tannins did not differ from the treated soybean meal with water and heat. Also the decrease of the amounts of solubilized N was established, on treating soybean meal with both tannin sources ($P < 0.01$). It is concluded that tannins extracted from *A. lebbekoides* and *A. cornigera* could be used for protecting of ruminal degradation to proteins of high biological value, as those of soybean meal.

Key words: ruminal degradation, fermentation, in vitro gas production, ammonia, rumen

Tropical pastures have low protein contents requiring the utilization of protein supplements for covering the requirements of growing animals or with high productive potential. However, the use of protein concentrates is almost prohibited, due to its effect on production costs and to the extensive protein degradation in the rumen, a process that leads to the inefficient use of proteins of high biological value, especially in animals with high protein requirements (Bunglavan and Dutta 2013).

On feeding with resistant ruminal degradation proteins, but digestible at the lower parts of the digestive tract, the growth rates and milk production of the animals are increased (Bunglavan and Dutta 2013). Conventional sources, as those of soybean meal (*Glycine max.*), are an excellent protein source, but with high ruminal degradability. This limits its contribution, qualitative or quantitative, in essential amino acids to the protein metabolized by the animals. Facing this condition, diverse methods have been developed to protect the proteins of this protein source of microbial degradation in the rumen and for increasing animal production (Soltan 2009). Among the treatments widely used at commercial scale is heat, reduction of sugars or the utilization of tannic acid.

Presently, there is great interest in the utilization of tannins as natural protectors of the proteins in ruminant rations. Tannins form stable complexes with the proteins, with ruminal pH, in a range of 3.5 – 7.0, but dissociate in the abomasum (pH lower than 3.5) (Getachew *et al.* 2000). This effect was demonstrated with the utilization of hydrolyzable tannins, condensed and raw extracts of these compounds (Lata *et al.* 2012 and Rodríguez *et al.* 2013a).

The objective of this experiment was to determine *in vitro* the potential of tannins extracted from two tropical shrub-like legumes (*Albizia lebbekoides* and *Acacia cornigera*) for the protection of the protein fraction of soybean meal and their effects on the fermentation indicators.

Materials and Methods

Preparation of evaluating treatments. Soybean meal ground at 1 mm particle size was used as protein substrate. As tannin sources, leaves and petioles of two tropical shrub-like legume species were collected: one of high tannin content (*A. lebbekoides*) and another with lower tannin contents, but with high reactivity of these compounds (*A. cornigera*) (Rodríguez *et al.* 2013a). Both shrub-like legumes were collected in November 2012 in the arboretum of the Institute of Animal Science of the Republic of Cuba. Legumes came from plants fully established in a typical red ferrallitic soil (Hernández *et al.* 1999), without irrigation or fertilization. Approximately 0.2 kg of fresh material was taken from each arboretum specimen. The collected material was grouped per species, dried in forced-air oven, with temperature regulated at 55° C for 72 h and later ground in a hammer mill at 0.5 mm particle size.

For tannin extraction, 6.25 g of each legume were weighed and placed in a precipitate glass with 100 mL of cold distilled water. Ascorbic acid (0.1 g) was added as antioxidant. Samples were agitated in an ultrasonic bath with cold water for 30 min at 10 min agitation intervals and 5 min rest. Later they were left to rest for 16 h and centrifuged at 3 000 x g for 15 min. The supernatant with the tannin extract of each legume was mixed with

25 g of soybean meal. The mixture was homogenized and left to rest 4 h. Also 25 g of soybean meal were moistened with 100 mL of distilled water. These three treatments were centrifuged at 3 000 x g for 15 min. The supernatant was eliminated and precipitates were dried in a forced-air oven, with controlled temperature at 55° C for 96 h combined to a fourth of 25 g of dry soybean meal.

The experimental treatments consisted of untreated soybean meal (1), soybean meal treated with tannin extract of *A. lebbekoides* and heat (2), soybean meal treated with tannin extracts of *A. cornigera* and heat (3), soybean meal treated with water and heat (4) and soybean meal treated with heat.

Experimental procedure. The *in vitro* technique of gas production in glass bottles described by Theodorou *et al.* (1994) was applied. From each treatment 800 mg were incubated in 100 mL bottles in culture medium without micromineral solution and resazurine. HCl-cysteine was used as reducing agent and an inoculum of ruminal microorganisms, in a 0.20 proportion of the total incubation volume (80 mL).

As inoculum was employed the ruminal contents of two rumen cannulated cows fed *ad libitum* grass forage supplemented with 1.0 kg of protein concentrate daily and free access to water and mineral salts. The ruminal content of each animal was collected before supplying the feed in the morning. Later, it was conserved in closed thermos until reaching the laboratory where it was filtered through various gauze layers and mixed the two inocula in equal proportions. During the process, inoculum temperatures were maintained at $39 \pm 1^\circ \text{C}$, and the anaerobic conditions through constant CO_2 flow. Bottles were sealed and incubated in a bath at controlled temperature (39° C). That moment was taken as zero hour of the incubation.

Gas production was measured every two hours until the 8 h of incubation, by means of a HD8804 manometer coupled to a TP804 (DELTA OHM, Italy) pressure gauge. After each measurement, the gas was released until equaling the external and internal bottle pressures. The gas volume was estimated from the pressure data obtained by a pre-established linear regression equation ($\text{gas (mL)} = (\text{pressure [103 Pa]} + 4.95)/2.5858$); $n = 132$; $r = 0.991$) (Rodríguez *et al.* 2013b). The gas volume was expressed per gram of organic matter incubated (OMinc).

At the end of incubation, the bottles were opened and their contents filtered through nylon bags (45 μm sieve size) for separating the solid phase of the incubation liquid. Samples of the filtrate were taken and preserved with a deproteinizing solution (2 % H_3PO_4) for the analysis of short chain fatty acids (SCFA), individual and with HCl (0.2 N) for determining ammonia. The preserved samples were conserved at -4 °C until further analysis.

Estimation of nitrogen solubilization (N). The

amount of N solubilized was estimated according to the procedure proposed by Raab *et al.* (1983), modified by Mota *et al.* (2005). This procedure was based on the N-NH₃ determination and gas production.

The N-NH₃ released, due to the microbial action in the nitrogenous compounds of the incubated substrates, is partially used for the microbial protein synthesis, while gas production (CO_2 and CH_4) is an indicator of the available energy for the protein synthesis. For estimating the relationship between gas production and the incorporation of N-NH₃ to the microbial protein, the substrate is incubated alone or with different starch levels. For that, the treatments evaluated in this study were also incubated with two levels of soluble starch (150 and 300 mg), at a rate of three bottles per treatment and starch level.

Solubilized N was calculated at 8 h of incubation, from the linear regression of the N-NH₃ concentration (“Y” axis, mg N-NH₃) versus gas production (“X” axis, mL gas). The intercept of the “Y” axis was considered as the amount of N-NH₃ released to the medium, when no energy was available (microbial synthesis zero). The difference between this value and the amount of N-NH₃, determined in the blank bottles, indicated the amount of solubilized N during the degradation process of the nitrogenous compounds in the evaluated substrates.

Chemical analysis. Individual SCFA concentration in the preserved samples was determined by gas chromatography, on injecting 0.5 μL , after centrifuging for 8 min the vials at 14 200 x g (Centrifuge ECEN-205, MRC Ltd., Hagsvish, Israel). A liquid-gas chromatograph DANI Master GC (DANI Instruments S.p.A. Milan, Italy) was used equipped with a capillary column DN-FFAP (length 30 m, internal diameter 0.32 mm, film thickness 0.25 μ) and a FID detector. H_2 was used a gas carrier and N_2 as auxiliary. Maximum temperature of the injector and detector was fixed at 200 and 250° C, respectively. Also total SCFA were obtained by algebraic sum of the individual SCFA determined. The quotient of the concentration of acetic and propionic acids (Ac/Pp relationship) were calculated. Ammonia analyses were carried out according to Conway (1957).

Experimental and statistical designs. An experimental random block design was applied. The incubations realized were considered as replications (4) and the means of the variables measured per treatment in each replicate were regarded as experimental unit. In each replication, four bottles per treatment and four blank bottles without substrate were incubated. Results from gas production at 8 h and the *in vitro* fermentation indicators were analyzed by ANOVA through the statistical package InfoStat (Di Rienzo 2010). When differences were found ($P < 0.05$), means of treatments were compared by the method of multiple comparisons proposed by Fisher (LSD Fisher) (Fisher and Yates 1958). Pearson’s correlation index was determined between the NH₃ levels produced and the estimated N

solubility.

Results and Discussion

Table 1 shows the effects of the treatments to which soybean meal was submitted on two indicators of the energetic metabolism, gas production and individual (acetic, propionic and butyric acids) and total SCFA at 8 h of incubation. There was no effect of the treatments on the production of total SCFA and only the treatment with heat treated soybean meal increased *in vitro* gas production ($P < 0.01$). Neither differences were found in individual SCFA productions nor in the acetic/propionic relationship. These results indicate that treatments applied to the soybean meal influenced negatively on the energy metabolism of ruminal microorganisms, since gas production or individual or total SCFA production were not decreased nor the relationship between C2:C3 fatty acids or the production profile of SCFA were modified. There was no decrease in the fermentability of the substrate by effect of the treatments with the tannin extracts or the physical processes of moistening and heat drying.

In other studies neither were there changes in the production of total SCFA, on evaluating the tannin effect of quebracho (*Schinopsis balansae*) on nutrient utilization in sheep fed a mixed diet of forage and concentrates (Attia *et al.* 2013) and in lactating cows supplemented with treated soybean meal with different procedures including the use of heat, extrusion and tannins (Soltan 2009). However, these results are in contradiction with those reported on evaluating other tannin sources as chemical additives, hydrolyzers or condensates. In these, there was a decrease of the amounts of SCFA and gas produced (El-Waziry *et al.* 2005, 2007, Mohammadabadi *et al.* 2010 and Mohammadabadi and Chaji 2012). These different effects are due to the fact that the magnitude of the action in the substrate fermentation depends on the chemical nature of the tannin used (e.g. structure, polymerization degree, reactivity) and their concentrations, as well as the interactions established with the nutrients and the

enzymes involved in the microbial metabolism (Barry *et al.* 1986 and Bueno *et al.* 2008).

It was also referred that on treating soybean meal with heat, the values of the kinetics parameters of *in situ* and *in vitro* degradation of the substrate are reduced (El-Waziry *et al.* 2005, 2007). This is attributed to the fact that feed heating can provoke the non-enzymatic protein glucosilation, by forming links among free-amino groups of the polypeptidic and aldehyde chains of carbohydrates through the Maillard reaction, decreasing the digestibility of the feeds. Results of this study indicate that if processes of this type occur, at least, their magnitude did not affect the fermentation indicators determined.

Regarding the nitrogen metabolism, figure 1, shows that tannins from albizia and acacia decreased the NH_3 levels at 8 h of incubation, respecting the untreated soybean meal ($P < 0.05$) although the NH_3 levels of the treatment with tannins from acacia did not differ from the values obtained with treated soybean meal with water and heat. The rest of the treatments (soybean meal treated with water and heat, soybean meal with heat) did not differ nor with regard untreated soybean meal. Thus, the effect of humidity or heat on protein degradability was discarded, two implicit factors in the treatment process of the protein source with tannins that could have influenced on the results.

In vitro decrease of the NH_3 levels on treating protein sources with tannins has been widely reported for soybean meal (El-Waziry *et al.* 2005, 2007 and Soltan 2009), sunflower (*Helianthus annuus*) (Mohammadabadi *et al.* 2010 y Mohammadabadi and Chaji 2012) and tannin extracts of *Acacia nilotica* in forage and concentrate mixtures (Lata *et al.* 2012). In *in vivo* conditions it was observed that on treating with tannins of chestnut bark (*Castanea sativa* Miller) a diet rich in concentrates for dairy cows the NH_3 levels in the rumen decreased compared to the untreated diet (Sliwinski *et al.* 2002). Similar results were reported in sheep fed a mixed diet of forage and concentrate (Attia *et al.* 2013) and on using small amounts of *Acacia cyanophylla* Lindl

Tabla 1. Effect of treatments used in gas production (GasP, mL g⁻¹ ini.OM) total and individual SCFA (mmoleL⁻¹), at 8 h of incubation

Treatment	PGas (ml g ⁻¹ MOinc)	Acetic acid (mmole L ⁻¹)	Propionic acid (mmole L ⁻¹)	Butyric acid (mmole L ⁻¹)	Total SCFA (mmole L ⁻¹)	Ac/Pp rate
Untreated soybean	61.21 ^a	60.33	19.08	9.18	91.61	3.20
Soybean + Albizia tannins + heat	54.89 ^a	61.40	19.48	9.35	92.56	3.32
Soybean + Acacia tannins + heat	51.94 ^a	56.58	18.24	8.36	85.67	3.25
Soybean + water + heat	53.04 ^a	52.82	17.39	7.37	79.43	3.24
Soybean + heat	71.96 ^b	53.19	16.92	8.22	80.84	3.26
SE	3.045**	6.990	2.427	1.242	11.093	0.076

^{ab} Means with different letters in the same column show significant differences ($P < 0.05$). ** ($P < 0.01$)

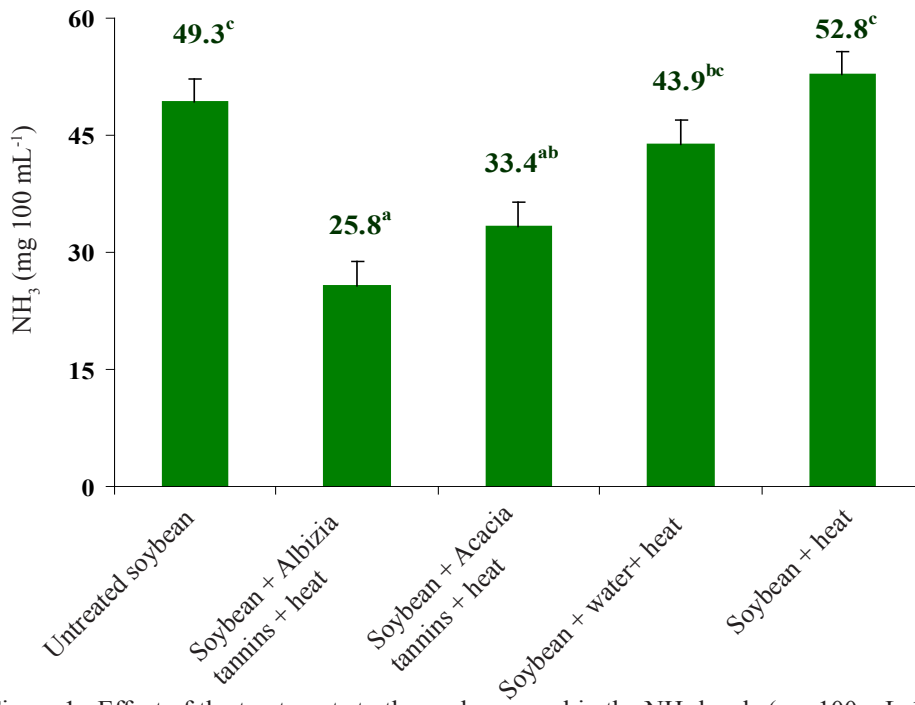


Figure 1. Effect of the treatments to the soybean meal in the NH₃ levels (mg 100 mL⁻¹) at 8 h of incubation (SE = ± 3.31; P < 0.05)

leaves, mixed with soybean meal in a protein supplement for sheep consuming oat hay (Ben Salem *et al.* 2005).

Figure 2 shows the amount of solubilized N of the proteins of the substrate by the action of ruminal microorganisms after 8 h of *in vitro* incubation. There was a decrease of the amounts of solubilized N, on treating soybean meal with both tannin sources and heat (P < 0.01), while the other treatments did not differ.

It must be highlighted that the NH₃ levels produced were closely related to the estimated N solubility

(r= 0.9375). They also confirmed that the reduction of the NH₃ levels in the treatments where the protein of the soybean meal was protected with tannins and heat was the product of the protecting action of these secondary compounds extracted from tropical legumes and not of the temperature to which they were submitted during the processing of the treated samples with these compounds. Similar results were observed on evaluating the effect of combining a tanniferous legume (*Lotus uliginosus* cv. Maku) with *Pannisetum clandestinum* in the *in vitro*

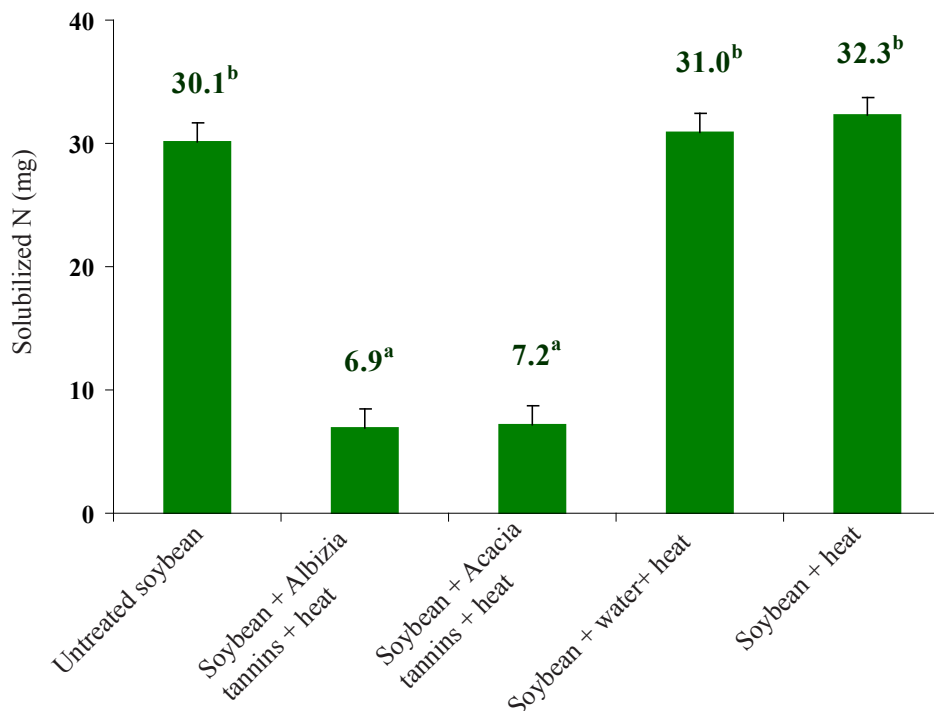


Figure 2. Effect of the evaluated treatments on the solubilized nitrogen (mg) of the soybean meal at 8 h of incubation (SE = ± 2.51; P < 0.05)

degradation of the proteins (Chipatecua *et al.* 2007).

Some studies indicate that the heat did not influence on the NH₃ concentration in the rumen of cows fed with treated soybean meal (Stern *et al.* 1985). However, other studies report that on treating soybean meal with heat decreases the NH₃ concentration in the rumen (El-Waziry *et al.* 2005, 2007). These contradictory results of the effect of heat on N solubility of the soybean meal proteins and the ruminal NH₃ concentration are probably due to differences in the temperatures and heating time, as well as to other peculiarities of the experimental procedures used.

The decrease of the protein solubility of soybean meal, the lower degradability of the proteins and, thus, the decrease of the NH₃ concentrations observed in the treatments with tannin extracts are the consequence of the action of these secondary compounds in the processes of the ruminal protein metabolism, mainly in the proteolysis, the peptide degradation and the amino acid deamination of the substrate (McSweeney *et al.* 2001).

There is experimental evidence of the tannin effects on the reduction of the N degradability of soybean meal in the rumen (Driedger and Hatfield 1972, Frutos *et al.* 2000 and El-Waziry *et al.* 2005, 2007). Hydrolyzable tannins (tanic acid) as the condensates (quebracho) reduce the protein degradability of soybean, mainly by lower degradation rhythm and by decrease of the fraction immediately degradable of the protein by effect of the formation of insoluble complexes of tannins-proteins (Getachew *et al.* 2008).

The effect of the tannins on ruminal fermentation of the substrates varies in function of the plant species and even the variety, which is related to its chemical composition (González *et al.* 2002 and Mohammadabadi and Chaji 2012). The similar effects of the two tannin sources evaluated on the N solubility of soybean meal in the rumen, in spite of the existing qualitative and quantitative differences between the tannins of both legumes (Rodríguez *et al.* 2013a), can be given because the activity of these secondary compounds in the ruminal fermentation is the result of the combined effects of the amount of these metabolites of the plant and its chemical reactivity in face of the substrates (Bunglavan and Dutta 2013).

Dentinho *et al.* (2007) indicated the importance of the tannin concentration in the extracts considering their linear relationship with the degradation rate and the effective protein degradation and of quadratic type with the soluble and non-soluble N fraction, but degradable. The majority of the authors suggest moderate dosages of these metabolites (Frutos *et al.* 2000 and El-Waziry *et al.* 2005, 2007), although others alert that the dosages vary in function of the tannin-protein relationship in the diet (Ben Salem *et al.* 2005). This aspect must be studied furtherly regarding the tannin sources evaluated in this study owing to the fact that very high concentrations of these secondary compounds can affect the enzymatic

activity of the ruminal microorganisms, even the cellulases, and of the enzymes in the small intestine (Makkar *et al.* 1988 and Attia *et al.* 2013).

It is important to highlight that tannin extraction in water was an adequate procedure for the objective of the study, since it allowed the extraction of sufficient amount of tannins (tannins were extracted from an amount of plant material equivalent to 25 % of the dry matter of the soybean meal to be treated) for protecting adequately an important protein fraction of soybean meal. Cold water was selected as solvent, since it is a solvent that can be use under practical production conditions. Lata *et al.* (2012) found that this solvent, the same as the methanol were excellent for tannin extraction regarding the use of other as hot water and acetone. It is concluded that the tannins extracted from *A. lebbekoides* and *A. cornigera* could be used for protecting the ruminal degradation to proteins of high biological value as those of soybean meal. New investigations are recommended for determining the optimum utilization dosages of these chemical additives. It is suggested to deepen on the practical possibilities of these plants for the chemical protection of soybean meal under production conditions. Further studies related to its effect on the productive indicators and N balance of the ruminants consuming them must be carried out.

References

- Attia, M.F.A., Nour El-Din, A. N., El-Shazly, K. A. & Sallam, S. M. 2013. Effect of quebracho tannins supplementation on nutrients utilization and rumen fermentation characteristics in sheep. *Alex. J. Agric. Res.* 58: 165
- Barry, T.N., Manley, T.R. & Duncan, S.J. 1986. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 4. Sites of carbohydrate and protein digestion as influenced by dietary reactive tannin concentration. *Br. J. Nutr.* 55: 123
- Ben Salem, H., Makkar, H.P.S., Nefzaoui, A., Hassayoun, L. & Abidi, S. 2005. Benefit from the association of small amounts of tannin-rich shrub foliage (*Acacia cyanophylla* Lindl.) with soya bean meal given as supplements to Barbarine sheep fed on oaten hay. *Anim. Feed Sci. Technol.* 122: 173
- Bueno, I.C.S., Vitti, D.M.S.S., Louvandini, H. & Abdalla, A.L. 2008. A new approach for *in vitro* bioassay to measure tannin biological effects based on a gas production technique. *Anim. Feed Sci. Technol.* 141: 153
- Bunglavan, S. J. & Dutta, N. 2013. Use of Tannins as Organic Protectants of Proteins in Digestion of Ruminants. *J. Livestock Sci.* 4: 67
- Chipatecua, M.R., Pabón, M.L, Cárdenas, E.A. & Carulla, J.E. 2007. Efecto de la combinación de una leguminosa tanífera (*Lotus uliginosus* cv Maku) con *Pennisetum clandestinum*, sobre la degradación *in vitro* de proteína y materia seca. *Rev. Col. Cienc. Pec.* 20: 40
- Conway, E.C. 1957. Microdiffusion analysis and volumetric error. 4th Ed. Crosby Lackwood & Sons, Ltd, London
- Dentinho, M.T., Moreira, O.C., Pereira, M.S. & Bessa, R.J. 2007. The use of a tannin crude extract from *Cistus ladanifer* L. to protect soya-bean protein from degradation

- in the rumen. *Animal*, 1: 645
- Di Rienzo, J.A., Casanoves F., Balzarini M.G., González L., Tablada M. & Robledo C.W. 2010. InfoStat versión 2010. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina
- Driedger, A. & Hatfield, E.E. 1972. Influence of tannins on the nutritive value of soybean meal for ruminants. *J. Anim. Sci.* 34: 465
- El-Waziry, A.M., Nasser, M.E.A. & Sallam, S.M.A. 2005. Processing methods of soybean meal. 1- Effect of roasting and tannic acid treated-soybean meal on gas production and rumen fermentation *in vitro*. *J. Appl. Sci. Res.* 1: 313
- El-Waziry, A.M., Nasser, M.E.A., Sallam, S.M.A., Abdallah, A.L. & Bueno, I.C.S. 2007. Processing Methods of Soybean Meal. 2. Effect of Autoclaving and Quebracho Tannin Treated-Soybean Meal on Gas Production and Rumen Fermentation *in vitro*. *J. Appl. Sci. Res.*, 3: 17
- Fisher, R.A. & Yates, F. 1958. *Statistical Tables for Biological, Agricultural and Medical Research*, Oliver and Boyd, Edinburg, UK
- Frutos, P., Hervás, G., Giráldez, F.J., Fernández, M. & Mantecón, A.R. 2000. Digestive utilization of quebracho-treated soya bean meal in sheep. *J. Agr. Sci.*, 134: 101
- Getachew, G., Makkar, H.P.S. & Becker, K. 2000. Effect of polyethelene glycol on *in vitro* degradability of nitrogen and microbial protein synthesis from tannin rich-browse and herbaceous legumes. *Br. J. Nutr.* 84: 73
- Getachew, G., Pittroff, W., Putnam, D.H., Dandekar, A., Goyal, S. & DePeters, E.J. 2008. The influence of addition of gallic acid, tannin acid, or quebracho tannins to alfalfa hay on *in vitro* rumen fermentation and microbial protein synthesis. *Anim. Feed Sci. Technol.* 140:444
- González, S., Pabón, M.L. & Carulla, J. 2002. Effects of tannins on *in vitro* ammonia release and dry matter degradation of soybean meal. *Arch. Latinoam. Prod. Anim.* 10:97
- Hernández, A., Pérez, J.M. & Bosch, D. 1999. Nueva versión de la clasificación genética de los suelos de Cuba. Ministerio de la Agricultura. Ciudad de la Habana, Cuba. p. 26
- Lata, M., Rai, S.N., Sharma, V.K., Jha, P., Jain, P. & Kumar, M. 2012. Effect of tannin from *Acacia nilotica* pods on *in vitro* and in sacco nutrient degradability and rumen fermentation pattern. *Indian J. Dairy Sci.* 65:382
- Makkar, H.P.S., Singh, B. & Dawra, R.K. 1988. Effect of tannin-rich leaves of oak (*Quercus incana*) on various microbial enzyme activities of the bovine rumen. *Br. J. Nutr.* 60:287
- McSweeney, C.S., Palmer, B., McNeill, D.M. & Krause, D.O. 2001. Microbial interactions with tannins: nutritional consequences for ruminants. *Anim. Feed Sci. Tech.* 91:83
- Mohammadabadi, T. & Chaji, M. 2012. The Influence of the plant tannins on *in vitro* ruminal degradation and improving nutritive value of sunflower meal in ruminants. *Pak. Vet. J.* 32: 225
- Mohammadabadi, T., Chaji, M. & Tabatabaei, S. 2010. The effect of tannin acids on *in vitro* gas production and rumen fermentation of sunflower meal. *J. Anim. Vet. Adv.* 9:277
- Mota, M., Rodríguez, R., Solanas, E. & Fondevila, M. 2005. Evaluation of four tropical browse legumes as nitrogen sources: Comparison of *in vitro* gas production with other methods to determine N degradability. *Anim. Feed Sci. Technol.* 123:341
- Cuban Journal of Agricultural Science, Volume 48, Number 3, 2014
- Raab, L., Cafantaris, B., Jilg, T. & Menke, K.H. 1983. Rumen protein degradation and biosynthesis. I. A new method for determination of protein degradation in rumen fluid *in vitro*. *Br. J. Nutr.* 50:569
- Rodríguez, R., de la Fuente, G., Gómez, S. & Fondevila, M. 2013a. Biological effect of tannins from different vegetal origin on microbial and fermentation traits *in vitro*. *Anim. Prod. Sci.* Available: <http://dx.doi.org/10.1071/AN13045>. [Consulted: 11/03/14]
- Rodríguez, R., Lores, J., Gutiérrez, D., Ramírez, A., Gómez, S., Elías, A., Aldana, A.I., Moreira, O., Sarduy, L. & Jay, O. 2013b. Inclusion of the microbial additive Vitafert in the *in vitro* ruminal fermentation of a goat diet. *Cuban J. Agric. Sci.* 47:171
- Sliwinski, B.J., Soliva, C.R., Machmuller, A. & Kreuzer, M. 2002. Efficacy of plant extracts rich in secondary constituents to modify rumen fermentation. *Anim. Feed Sci. Technol.*, 101: 101
- Soltan, M.A. 2009. Rumen fermentation characteristics and lactation performance in dairy cows fed different rumen protected soybean meal products. *Pak. J. Nutr.* 8:695
- Stern, M.D., Santos, K.A. & Satter, L.D. 1985. Protein degradation in rumen and amino acid absorption in small intestine of lactating dairy cattle fed heat-treated whole soybeans. *J. Dairy Sci.* 68:45
- Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B. & France, J. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim. Feed Sci. Tech.* 48:185

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