# Effects of tannins on *in vitro* ammonia release and dry matter degradation of soybean meal*

**S. González[1], M. L. Pabón and J. Carulla[2]****

[1]Departamento de Química, Facultad de Ciencias. [2]Facultad de Medicina Veterinaria y de Zootecnia.
Universidad Nacional de Colombia, Bogotá, Colombia

**ABSTRACT:** The efficiency of quebracho (*Schinopsis* spp), acacia (*Acacia* spp) and chestnut (*Castanea* spp.) tannins to protect protein of soybean meal from *in vitro* degradation was determined. Each tannin was added to soybean meal at levels of 0, 2, 4, and 8% of dry weight and incubated by triplicate in ruminal liquor and McDougall buffer for 48 h (degradation by rumen bacteria) or 48 h plus 24 h of pepsin digestion (degradation by rumen bacteria plus pepsin). *In vitro* ammonia, isobutyric and isovaleric acid production decreased by addition of tannins to soybean meal. Reduction of ammonia and isobutyric acid per unit of tannin added (slope) was higher ($p<0.05$) for chestnut than for quebracho or acacia tannins. *In vitro* DM degradation (DMD) by rumen bacteria plus pepsin decreased when tannins were added to soybean meal. Decrease in DMD by rumen bacteria per unit of tannin added (rate) was higher and decrease in DMD by rumen bacteria plus pepsin was lower for chestnut tannin than for quebracho or acacia tannin ($p<0.05$). Results suggest that chestnut tannins are more efficient in protecting soybean meal from *in vitro* degradation by rumen bacteria with the lowest negative effect on *in vitro* rumen plus pepsin degradation as compared to acacia or quebracho tannins.

Key words: Polyphenols, undegradable protein, condensed tannins, hydrolysable tannins

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# Efectos de los taninos en la degradación *in vitro* de la materia seca y la concentración de amoniaco de la torta de soya

**RESUMEN:** Se determinó la eficiencia de los taninos quebracho (*Schinopsis* spp.), acacia (*Acacia* spp.) y castaño (*Castanea* spp) para proteger la torta de soya de la degradación en un sistema *in vitro.* Se adicionó tanino (0, 2, 4 y 8% del peso seco) y se incubó en triplicado con fluido ruminal y amortiguador de McDougall durante 48 h (degradación por bacterias ruminales) o 48 h más 24 h de digestión con pepsina (degradación por bacterias ruminales más pepsina). La reducción de amoniaco y ácido isobutírico por unidad de tanino adicionado (pendiente) fue mayor ($p<0.05$) para el castaño que para el quebracho o la acacia. La degradación *in vitro* de la materia seca por bacterias ruminales más pepsina disminuyó cuando los taninos fueron adicionados a la torta de soya. Cuando se comparó el castaño con el quebracho y la acacia, la disminución en la degradación de materia seca por bacterias ruminales por unidad de tanino adicionado (tasa) fue mayor mientras la disminución en la degradación de materia seca por bacterias ruminales más pepsina fue menor ($p<0.05$). Los resultados sugieren que los taninos de castaño son más eficientes que los de acacia o quebracho para proteger la torta de soya de la degradación *in vitro* por las bacterias ruminales con el menor efecto negativo en la degradación con bacterias ruminales más pepsina.

Palabras claves: Polifenoles, proteina no-degradable, taninos condensados, taninos hidrolizables

## Introduction

Addition of tannins to ruminant diets may improve efficiency of nitrogen utilisation. Some studies have shown an increase of amino acid flow to the duodenum and amino acid absorption when tannins are included in diets (Jones and Mangan, 1977, Egan and Ulyatt, 1980, Waghorn *et al*., 1987). Other experiments have demonstrated that tannins

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E-mail: djcarull@veterinaria.unal.edu.co / mpabon@multi.net.co
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**To whom correspondence should be addressed. Phone 3165471 Fax 3165401

reduce intake, protein and dry matter digestibility thereby decreasing animal performance (Barry and Duncan, 1984, Reed *et al*., 1990, Dawson *et al*., 1999). Differences in results among trials may be related to the nature and concentration of tannins used. A tannin source that would decrease ruminal protein degradation without affecting protein digestion in the intestine would be ideal in diets for ruminants.

The objective of this experiment was to examine the effects of three types of semipurified tannins (i.e., quebracho, acacia or chestnut) on *in vitro* degradation of soybean meal.

## Materials and Methods

**Soybean meal and tannins.** Soybean meal and semipurified commercial tannins were used. They were quebracho (*Shinopsis* spp.), acacia (*Acacia* spp) and chestnut (*Castanea* spp.) produced by S.A. Ledoga (Italy). Soybean meal and tannins were analyzed for moisture (AOAC-7.007, 1984), ash (AOAC-7.009, 1984) and neutral detergent fiber according to Van Soest *et al.* (1991) without sodium sulphite, alpha amylase or residual ash for tannins but adding amylase to soybean meal. Soybean meal was analysed for crude protein (AOAC-7.033-7.037, 1984) and soluble nitrogen according to Van Soest *et al.* (1995). Total phenol and condensed tannins of semipurified tannins were determined using the Prussian Blue method (Price and Buttler, 1977) and butanol/HCl method respectively (Porter *et al*., 1986).

**Soybean meal and tannin mixtures.** Soybean meal (0.5 g) was weighed in 100 mL plastic tubes and 0.01, 0.02 or 0.04 g (2, 4, or 8%) of one of the tannin sources (i.e., acacia, quebracho or chestnut) dissolved in McDougall buffer (5 mL) was added. Triplicate tubes were used for each tannin level and for the control (no tannin added). Tubes were placed in a water bath at 39°C for 12 h to allow tannin and protein to react.

***In vitro* incubation.** The Tilley and Terry (1963) procedure was used to determine degradation by rumen bacteria and degradation by rumen bacteria plus pepsin.

*Degradation by rumen bacteria.* Ruminal fluid was collected from two steers grazing kikuyo (*Pennisetum clandestinum*) and clover (*Trifolium repens*), filtered through cheesecloth and kept at 39°C under $CO_2$ for 30 minutes. After this time, 50 mL of diluted ruminal fluid (ruminal fluid:McDougall buffer 1:4) were added to tubes that contained soybean meal or tannin treated soybean meal. After gasifying with $CO_2$, tubes were sealed with rubber stoppers provided with bunsen valves and incubated at 39°C for 48 h, shaking periodically. Ammonia, *in vitro* DM degradation (IVDMD) and isobutyric and isovaleric acid were determined in the fluid from the incubation tubes. A different incubation trial was performed for each response variable. Samples used to determine ammonia release contained McDougall buffer without urea. To ensure that decreases in IVDMD and VFA was not due to nitrogen shortage, urea was added to McDougall buffer according to the original procedure of Tilley and Terry (1963).

*Degradation by rumen bacteria plus pepsin.* After ruminal incubation, 6 mL of 7% HCl w/v and 2 mL of a 5% pepsin solution were added to each tube. Samples were incubated for additional 24 h at 39°C and the content of each tube was vacuum filtered in order to determine dry matter degradation.

**Chemical analysis.** Aliquots of 4 mL were taken from each ruminal incubation tube and acidified with 80 µL of $H_2SO_4$ (80% v/v). Aliquots of 1 mL were centrifuged at 9.000 x g for 10 min. Ammonia concentration was determined in the supernatant using a colorimetric method (McCullough, 1967).

For VFA determination, a 1 mL aliquot was taken from each ruminal incubation tube and frozen for 72 h. After thawing, 250 µL of a solution containing 2 ethylbutyric acid (25mM) and metaphosphoric acid (25%) were added. After 30 min, tubes were centrifuged at 9000 x g for 10 min, filtered through 0.45 µm millipore membranes and an aliquot of 0.8 µL injected in a gas chromatograph (Shimadzu GC14A equipped with a flame ionization detector (FID), coupled to an integrator (Shimadzu CR4) and fitted with a glass column of 1.0 m length, 3 mm inner diameter packed with SP 1220 80/100 Chromosorb $H_3PO_4$ 1%). Nitrogen, 99.99% was used as a carrier and hydrogen and dried air were used for the FID.

**Statistical analysis.** Linear and quadratic regression equations were calculated using ammonia, isobutyric or isovaleric acid concentrations, rumen bacteria DM degradation or rumen bacteria plus pepsin DM degradation as dependent variables (Y) and proportion of added tannin as the independent variable (X). Determination coefficients for linear and quadratic relationships were calculated and the higher determination coefficient was chosen. Statistical differences among slopes of the treatments were tested using a paired Student´s t test. ANOVA analysis was performed and differences among means were tested using Tukey´s test in SAS (Snedecor and Cochran, 1980).

## Results

**Characterization of soybean meal and tannin sources.** The composition of soybean meal and tannin sources is presented in Table 1. Total phenol content followed the order chestnut>quebracho>acacia, but quebracho contained mainly condensed tannins and chestnut hydrolysable tannins.

**Protein degradation.** Ammonia, isobutyric and isovaleric acid concentration decreased with addition of tannins to soybean meal (Figures 1 and 2). Reduction of ammonia and isobutyric acid per unit of tannin added was higher for chestnut tannins than for quebracho or acacia tannins ($p<0.05$) but isovaleric acid was not affected (Table 2).These results showed that chestnut tannins were more efficient in decreasing *in vitro* protein degradation.

Table 1. Composition of soybean meal and tannin sources.

| | Soybean meal | Tannin sources | | |
|---|---|---|---|---|
| | | Acacia | Quebracho | Chestnut |
| DM (%) | 87.8 | 88.5 | 91.4 | 86.9 |
| CP (%) | 50.2 | - | - | - |
| Soluble protein (%) [1] | 23.0 | - | - | - |
| NDF (%) | 12.3 | - | - | - |
| OM (%) | 80.5 | 95.1 | 91.2 | 97.2 |
| Total phenols (%) [2] | - | 33.2 | 39.3 | 47.2 |
| Condensed tannins (A550/mg) | - | 1.13 | 0.82 | 0.05 |
| Hydrolysable tannins (%) [2] | - | 8.2 | 0 | 24 |

1.% of CP
2. As gallic acid

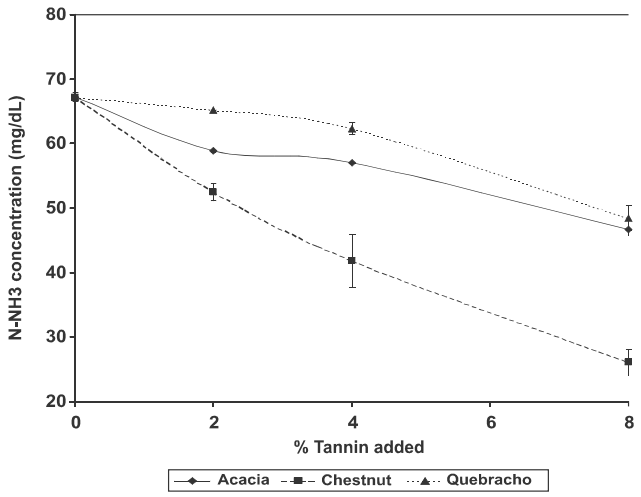


Figure 1. Changes in ammonia concentration after addition of semipurified tannins (acacia, quebracho or chesnut) to soybean meal in an *in vitro* incubation system.

***In vitro* degradation.** *In vitro* and *in vitro* plus pepsin DM degradation decreased, when any tannin was added to soybean meal (Figures 3 and 4). Decreases in ruminal DM degradation per unit of tannin added was higher ($p<0.05$) for chestnut tannin than for quebracho, which was higher ($p<0.05$) than for acacia tannin. Decreases in ruminal plus pepsin DM degradation per unit of tannin was lower ($p<0.05$) for chestnut tannin than for quebracho or acacia tannin (Table 2).

## Discussion

Several experiments *in vivo* and *in vitro* have shown a decrease in ruminal protein degradation by addition of tannins (Zelter and Leroy, 1966, Driedger and Hatfield; 1972, Barry and Manley; 1984, Waghorn *et al*., 1987). Tannin-protein interactions have also been determined using a

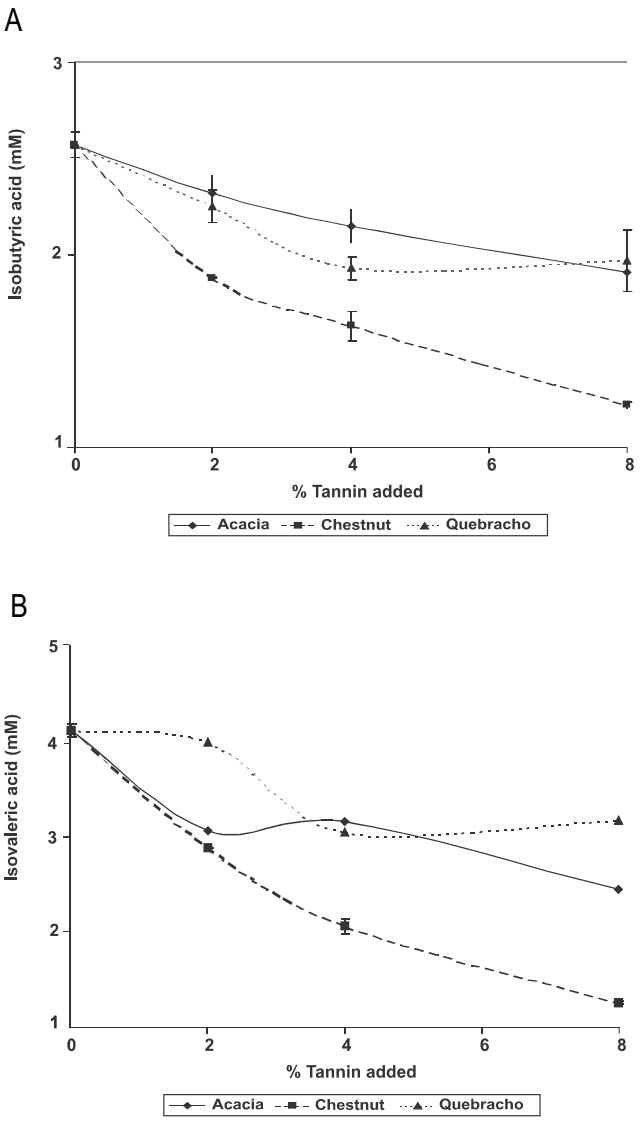


Figure 2. Changes in isobutyric (A) and isovaleric acid (B) after addition of semipurified tannins (acacia, quebracho or chesnut) to soybean meal in an *in vitro* incubation system.

Table 2. Effects of tannins on *in vitro* degradation of soybean meal[1].

| | Acacia | Quebracho | Chestnut |
|---|---|---|---|
| **Ammonia** | | | |
| Intercept (mg N-$NH_3$ x $dL^{-1}$) | 66.28 | 68.49 | 65.25 |
| Regression Coef. ($\beta_1$, mg N-$NH_3$ x $dL^{-1}$ x % $tannins^{-1}$) | -2.48[a] | -2.28[a] | -5.14[b] |
| $R^2$ | 0.95 | 0.89 | 0.49 |
| **Isobutyric acid** | | | |
| Intercept (mM) | 2.57 | 2.58 | 2.54 |
| Regression Coef. ($\beta_1$, mM x % $tannins^{-1}$) | -0.13[a] | -0.23[a] | -0.32[b] |
| ($\beta_2$, mM x (% $tannins^2$)$^{-1}$) | 0.006 | 0.02 | 0.02 |
| $R^2$ | 0.69 | 0.86 | 0.97 |
| **Isovaleric acid** | | | |
| Intercept (mM) | 4.02 | 4.26 | 4.12 |
| Regression Coef. ($\beta_1$, mM x % $tannins^{-1}$) | -0.34 | -0.33 | -0.67 |
| ($\beta_2$, mM x (% $tannins^2$)$^{-1}$) | 0.01 | 0.03 | 0.04 |
| $R^2$ | 0.60 | 0.48 | 0.90 |
| **IVDMD[2]** | | | |
| Intercept (% DMD) | 70.16 | 71.53 | 70.59 |
| Regression Coef. ($\beta$, % DM x % $tannins^{-1}$) | -0.86[a] | -0.61[b] | -3.17[c] |
| $R^2$ | 0.74 | 0.73 | 0.96 |
| **IVDMD plus pepsin[2]** | | | |
| Intercept (% DMD) | 88.49 | 87.80 | 88.53 |
| Regression Coef. ($\beta$, % DMD x % $tannins^{-1}$) | -1.19[a] | -1.37[a] | -0.46[b] |
| $R^2$ | 0.90 | 0.47 | 0.79 |

1.Values in a row that do not share a common letter differ significantly ($p<.05$).

2. IVDMD, in vitro Dry Matter Digestibility.

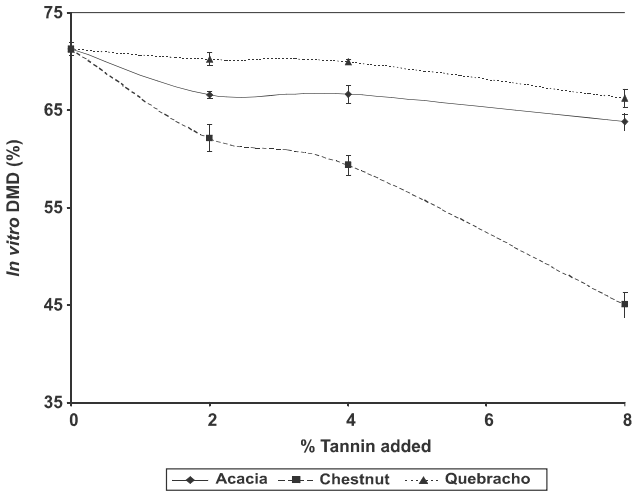


Figure 3. Effect of semipurified tannins (acacia, quebracho or chestnut) on *in vitro* DMD degradation.

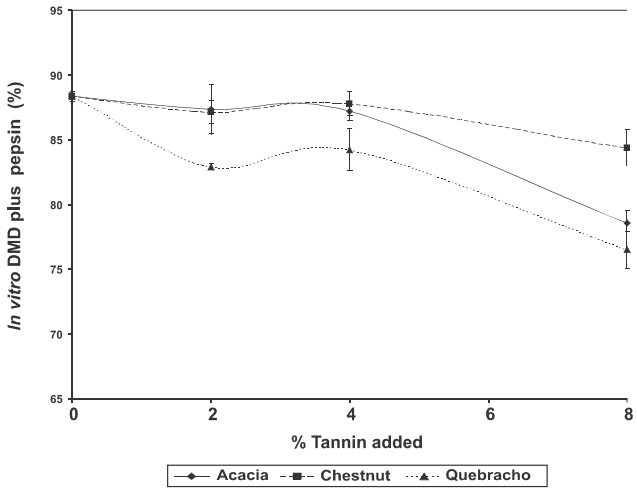


Figure 4. Effect of semipurified tannins (acacia, quebracho or chestnut) on *in vitro* DMD degradation plus pepsin of soybean meal.

competitive binding assay (Asquith and Butler, 1986) and with the radial diffusion method indicating that the capacity of tannins to bind proteins (astringency) are specific for each tannin and depends on the type of protein. However, only a few experiments have compared the effects of tannins of different chemical nature on *in vitro* ruminal degradation.

We have tested the effects of different types of tannins on an *in vitro* system using 48 h as incubation time. Other researchers have suggested that to estimate protein degradation shorter incubation times and an inhibitor of both deamination and ammonia uptake should be used (Broderick, 1978, Broderick, 1987). Our system does not consider the limitations of interpretation associated with microbial synthesis and incorporation of ammonia to the bacteria at longer incubation times. In addition, the substrate (soybean meal) was the same for all treatments. The results showed that the system may not be sensitive to the effects of adding different types of tannins. This system may not be accurate to measure protein degradability but it can be used to compare the biological activity of different types of tannins in an *in vitro* system.

We found that chestnut tannins are more efficient in decreasing protein and DM degradation by rumen bacteria. These tannins had the highest total phenol and hydrolysable tannin and the lowest condensed tannin content (Table 1). It could be argued that percentaje of total phenols could explain the differences among tannin sources. However, when

treatments were adjusted for total phenols, chestnut tannins were still more efficient ($p<0.05$), suggesting that the type of tannin should be considered. Interactions between proteins and tannins occur mainly by hydrogen bridges formed between OH groups of phenols and $NH_2$, SH, OH and CO of proteins (Siebert, 1996). Hydrolysable tannins have more OH groups than condensed tannins (Hagerman and Butler, 1991) suggesting that the major proportion of this type of tannin in chestnut may explain the differences among sources.

Several authors have suggested that tannin-protein interactions will be weakened at abomasal pH (Jones and Mangan, 1977) so that there is no negative effects of tannins on protein digestibility. Driedger and Hattfield (1972) found that DM digestibility of soybean meal at pH 7.0 decreased by addition of tannic acid while abomasal digestibility was not affected. In our experiment, at *in vitro* plus pepsin conditions there was still a negative effect on DM digestibility of soybean meal when tannins were added, suggesting that some tannin remains bound. This is in agreement with *in vivo* studies that showed that feeding tannins to sheep increased fecal nitrogen excretion (Barry *et al* 1986). The depression in dry matter digestibility after acid-pepsin addition was less for chestnut tannins (mainly hydrolyzable tannins) than for the other tannin sources (Table 2). This suggests that binding between soybean meal protein and hydrolyzable tannins is weaker under abomasal conditions than for condensed tannins perhaps due to interactions different from hydrogen bonds for these tannins.

## Conclusions

Our results showed that chestnut tannins are more efficient in protecting soybean meal from *in vitro* degradation by rumen bacteria with the lowest negative effect on *in vitro* rumen bacteria plus pepsin degradation as compared to acacia or quebracho tannins. Although in vitro results can not be extrapolated to the whole animal, we suggest that chestnut tannins could have a beneficial effect *in vivo* by increasing rumen escape protein but VFA and microbial ruminal protein formation could be depressed.

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