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Use of tannins to protect insect meal protein against ruminal degradation in sheep

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Abstract. Due to the high dependence of ruminant feeding systems on the use of soybean meal as source of protein, insect meals have been suggested as a promising alternative. However, a high rumen degradability might be a drawback for its utilisation. In this regard, tannins are known to be able to exert beneficial effects on the digestive utilization of protein-rich feeds due to a reduction of their degradation in the rumen, especially of the protein, although they might also restrict intestinal digestibility. Therefore, this experiment was conducted to examine if tannins could improve the digestive utilization of 4 insect meals (from *Acheta domesticus*, *Alphitobius diaperinus*, *Tenebrio molitor* and *Zophobas morio*). To this aim, four ruminally cannulated sheep were used to study the ruminal degradation and intestinal digestibility of these feedstuffs as well as those of soybean meal, which was used as a reference. The five substrates were treated with either 0% (control) or 15% DM of commercial tannin extracts from oak (OAK) or quebracho (QUE), and incubated *in situ* for 16 h. Afterwards, the intestinal digestibility of non-degraded protein was estimated *in vitro*. Both oak and quebracho tannins were able to protect the protein of insect meals from ruminal degradation, following a similar pattern than that observed in soybean meal. On average, reductions were about 15%, with no significant differences between oak and quebracho effects. On the opposite, the OAK treatment might offer advantages over QUE in terms of intestinal protein digestibility, as the latter caused a lower value in *A. domesticus* compared with the control. More research would be advisable to confirm these results, particularly *in vivo*.

Keywords. Alternative feeds – Condensed tannins – Hydrolysable tannins – Digestive utilisation.

Utilisation de tanins pour protéger la protéine de farine d'insectes contre la dégradation ruminale chez les ovins

Résumé. *En raison de la forte dépendance à l'utilisation de tourteau de soja comme source de protéines dans les systèmes d'alimentation des ruminants, la farine d'insectes a été suggérée comme une alternative prometteuse. Cependant, une forte dégradation de sa protéine dans le rumen pourrait constituer un inconvénient à son utilisation. À cet égard, les tanins peuvent avoir des effets bénéfiques sur l'utilisation digestive des aliments riches en protéines, en réduisant leur dégradation dans le rumen, même si ils peuvent limiter la digestibilité intestinale. Par conséquent, cet essai a été mené afin d'examiner si les tanins pouvaient améliorer l'utilisation digestive de 4 farines d'insectes (provenant d'*Acheta domesticus, Alphitobius diaperinus*, Tenebrio molitor *et* Zophobas morio*). Pour ce faire, quatre moutons munis d'une canule ruminale ont été utilisés afin d'étudier la dégradation ruminale et la digestibilité intestinale de ces aliments, en comparaison avec celles du tourteau de soja (aliment de référence). Les cinq substrats ont été traités avec 0 (témoin) ou 15% MS des extraits commerciaux de tanin de chêne (OAK) ou de quebracho (QUE) et par la suite ils ont été incubés in situ pendant 16 h. Ensuite, la digestibilité intestinale de la protéine non dégradée a été estimée in vitro. Les tanins de chêne et de quebracho ont permis de protéger la protéine des farines d'insectes de la dégradation ruminale, en suivant une tendance similaire à celle observée pour le tourteau de soja. En moyenne, la réduction de la dégradation protéique a été d'environ 15%, sans différence significative entre les effets du chêne et du quebracho. Au contraire, en termes de digestibilité intestinale des protéines, le traitement OAK pourrait offrir des avantages par rapport à QUE, puisque ce dernier a entrainé une valeur inférieure pour A. domesticus par rapport au contrôle. D'autres recherches seraient requises pour confirmer ces résultats, particulièrement en conditions* in vivo.

Mots-clés. *Aliments alternatifs – Tanins condensés – Tanins hydrolysables – Utilisation digestive.*

I – Introduction

Ruminant feeding systems are highly dependent on soybean meal as source of protein. This dependence is especially high in Europe due to the ban on the inclusion of meat and bone meals and their derivatives in ruminant diets, which obliges to employ almost exclusively protein of vegetable origin. Although the use of insects has been suggested as an alternative source of protein for livestock (Makkar *et al*., 2014), to date there are very few reports characterizing this type of protein feeds in ruminants (Jayanegara *et al*., 2017; Rashmi *et al*., 2018). No data on the extent of ruminal degradation of insect meal protein seems to be available in the literature.

In addition, it is known that tannins (both hydrolysable and condensed) can reduce the rumen degradation of dietary protein and thus improve the supply of amino acids susceptible of being absorbed in the intestine (Frutos *et al*., 2000; Hervás *et al*., 2000; Makkar, 2003). However, we are not aware of studies applying this treatment to protect insect meals.

Therefore, this trial was conducted to examine if tannins could reduce the ruminal degradation of insect meal protein without affecting its intestinal digestibility, thus improving its digestive utilization. To this aim, different insect meals were treated with oak and quebracho tannin extracts, and compared with soybean meal as a reference feed.

II – Materials and methods

Four ruminally cannulated Merino sheep (57.4 kg of body weight) were fed a total mixed ration (60:40 forage:concentrate ratio, 91.6% DM, 20.5% CP, 31.4% NDF) at 1.1 times their estimated maintenance energy requirements (approx. 900 g/animal and day).

Four insect meals were studied: (1) mealworms (*Tenebrio molitor*); (2) morioworms (*Zophobas morio*); (3) buffaloworms (*Alphitobius diaperinus*), and (4) adult domestic crickets (*Acheta domesticus*). In addition, soybean meal was used as a reference feed. Their chemical composition is reported in Table 1.

Table 1. Chemical composition of the meals[1]

	DM	OM	CP	NDF	ADF	EE	Starch
Soybean	87.5	93.1	50.6	14.5	9.3	3.5	0.3
Tenebrio molitor	93.2	96.6	50.9	19.5	7.6	34.4	4.5
Zophobas morio	93.7	96.6	37.8	9.6	5.3	48.8	1.8
Alphitobius diaperinus	93.3	96.0	64.7	11.4	7.3	24.7	0.9
Acheta domesticus	91.3	94.7	69.9	13.4	8.4	18.1	1.6

[1]DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; EE: ether extract. Results are expressed as % DM, except DM, which is expressed as % fresh matter.

Fifty g of each meal were ground (2 mm) with dry ice and treated with 15% DM of tannin extracts (Frutos *et al*., 2000; Hervás *et al*., 2000) by spraying 30 mL of distilled water with 7.5 g of a commercial tannin extract of oak (OAK, >65% tannins) or quebracho (QUE, ≥69% tannins), or with tannin-free water (Control treatment). The substrates were then dried at 45 ºC for 48 h.

The ruminal degradation of the meals was studied using the nylon bag technique (Ørskov and McDonald, 1979). These bags (50 μm pore size, R1020, Ankom Technology, USA) were filled with 6 g of substrate and incubated for 16 h in the rumen of the cannulated sheep. After removal from the rumen, the bags were washed with cold tap water and frozen (-30 ºC) for 24 h. Once defrosted, bags were washed again with cold water in a washing machine and dried in a forced-air oven at 45 ºC for 48 h to estimate DM disappearance (DMD). The residues were ground to 1 mm and CP concentration was analysed (ISO 5983-2:2009) to determine CP disappearance (CPD). The in-

testinal digestibility of the non-degraded protein (IDNDP) of each substrate was determined following the *in vitro* technique described by Calsamiglia and Stern (1995).

For each feed, results were submitted to ANOVA using the MIXED procedure of SAS (v9.4, SAS Inst. Inc., USA) with a model that included the fixed effect of treatment and the random effect of animal. Means were adjusted for multiple comparisons using Bonferroni's method.

III – Results and discussion

The treatment of soybean meal with tannins decreased CPD compared with the untreated meal (Table 2). The mean reduction was 15% and, although there were no significant differences between OAK and QUE, CPD was numerically lower in the first treatment, which would help explaining the reduction in DMD ($P<0.05$). This response was expected based on the known effect of tannins protecting the protein from ruminal degradation (Frutos *et al*., 2000; Dentinho *et al*., 2014). Similarly, it was predicted that the dose of tannins would not detrimentally affect the intestinal digestibility of non-degraded protein of this reference feed, although the use of higher doses or other tannin extracts might reduce it (Frutos *et al*., 2000; Hervás *et al*., 2000; Dentinho *et al*., 2014). Intestinal digestibility tended to be slightly higher in OAK than in QUE, but there were no significant differences compared with the control.

Table 2. Disappearances (g/g) of dry matter (DMD) and crude protein (CPD) after 16 h of incubation in the rumen, and intestinal digestibility (g/g) of the non-degraded protein (IDNDP) of feeds treated or not with 15% of a tannin extract from oak (OAK) or quebracho (QUE)

	Treatment				
	Control	OAK	QUE	SED[1]	Prob.[2]
Soybean meal					
DMD	0.893[a]	0.790[b]	0.837[ab]	0.0278	0.017
CPD	0.914[a]	0.736[b]	0.812[b]	0.0318	0.002
IDNDP	0.680	0.721	0.644	0.0234	0.062
Tenebrio molitor					
DMD	0.654	0.632	0.643	0.0107	0.190
CPD	0.486[a]	0.390[b]	0.417[b]	0.0094	<0.001
IDNDP	0.782[ab]	0.796[a]	0.723[b]	0.0234	0.028
Zophobas morio					
DMD	0.839[a]	0.786[b]	0.797[b]	0.0126	0.005
CPD	0.724[a]	0.574[b]	0.596[b]	0.0161	<0.001
IDNDP	0.703[b]	0.780[a]	0.702[b]	0.0157	0.001
Alphitobius diaperinus					
DMD	0.789[a]	0.714[b]	0.684[b]	0.0146	<0.001
CPD	0.778[a]	0.648[b]	0.620[b]	0.0176	<0.001
IDNDP	0.640[b]	0.780[a]	0.645[b]	0.0127	<0.001
Acheta domesticus					
DMD	0.702	0.679	0.680	0.0134	0.201
CPD	0.702[a]	0.635[b]	0.632[b]	0.0145	0.003
IDNDP	0.728[a]	0.757[a]	0.645[b]	0.0242	0.005

[a,b]Within a row, different superscripts indicate significant differences due to treatment ($P<0.05$).

[1]Standard error of the difference. [2]Probability.

The CPD of insect meals followed the same pattern of variation as in the soybean meal, with OAK and QUE reducing its value in all cases (P<0.05). Decreases ranged from 10% in *A. domesticus* to 19% in *Z. morio*, but no apparent relationship was observed between the magnitude of the response and the initial CP value of each insect or its CPD in the control. This latter parameter was relatively low in *T. molitor*, which would limit the interest of using tannin extracts to further decrease it. Consistent with soybean meal, results of CPD may also explain changes in DMD in *Z. morio* and *A. diaperinus* (-6 and -11%, respectively). We are not aware of other publications on the protection of insect protein against ruminal degradation.

Similarly, in the absence of available data in the literature, the effect of tannins on the intestinal digestibility of insect protein can only be compared with vegetable protein-rich feeds. The IDNDP of insects was similar or higher than that of soybean meal and, as indicated for the latter feed, it was higher in OAK than in QUE for all of them (P<0.05) and, surprisingly, also than in the control for *Z. morio* and *A. diaperinus*. Although previous studies had shown reductions or no effects of tannins on intestinal digestibility (Hervás *et al*., 2000; Makkar, 2003; Dentinho *et al*., 2014), it could be speculated that differences between OAK and QUE in the breakdown of tannin-protein complexes as well as a partial degradation in the rumen of the hydrolysable tannins from oak might help to explain our results. In any event, although OAK could be suggested to be more advantageous than QUE to improve the digestive utilization of insect protein, further studies must be conducted to confirm our observations, particularly *in vivo*. The economic cost of each extract must also be evaluated before selection. Similarly, additional research would be advisable to examine the responses to lower tannin doses, extracts of lower purity or from different origins, with the ultimate goal of minimising costs under practical conditions. Finally, given the high fat content of some insects (Table 1), it would be of great interest to characterize the effect of these nutritional strategies on ruminal lipid metabolism, because some tannins have been shown to favour the ruminal accumulation of certain desirable fatty acids (e.g., linoleic, α-linolenic and vaccenic acids, Toral *et al*., 2018).

IV – Conclusions

Treating insect meals with 15% of tannin extracts from oak or quebracho is effective to protect their protein from ruminal degradation. Concerning the intestinal digestibility of non-degraded protein, oak tannins seem more advantageous than those of quebracho in some cases.

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