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in

Ruiz R. (ed.), López-Francos A. (ed.), López Marco L. (ed.).
Innovation for sustainability in sheep and goats

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 123

2019
pages 177-181

Effect of tannins on indoles content and "pastoral" flavor of lamb meat

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Abstract. Skatole and indole derive from the degradation of tryptophan in the digestive tract and are responsible for off-flavors in sheep's meat and fat. Their formation and absorption can be controlled through feeding strategies. Particularly, dietary polyphenols reduce the biodegradation of tryptophan and are claimed to mitigate the "pastoral" flavor of sheep meat. The experiment aimed at evaluating the effect of the polyphenols nature on sensory and chemical characteristics of lamb meat. Fifty-three lambs of 70 days of age were divided in six groups of 7-10 animals each and fed the experimental diets for 75 days. The control group received a diet formulated with barley, bran, dehydrated alfalfa and soybean meal. The remaining groups were fed the control diet with the following integrations: i) 15% cardus, in partial substitution of dehydrated alfalfa; ii) 4% tara tannin extract (hydrolysable tannins – gallic acid), iii) 4% chestnut tannin extract (hydrolysable tannins – ellagic acid), iv) 4% mimosa tannin extract (condensed tannins-profisetinidin) and, v) 4% gambier tannin extract (condensed tannins-catechin). Perirenal fat was extracted in methanol and passed through a Sep-Pak C18 column for analysis of fatty acids profile. Skatole and indole content was quantified using an HPLC system with a fluorescence detector. A trained panel was asked to carry out the Quantitative Descriptive Analysis of sheep meat samples from *longissimus dorsi* muscle as well as an olfactory evaluation of fat samples. Diets rich in hydrolysable tannins significantly reduced "pastoral" flavour of perirenal fat, and at the same time demonstrated greater efficacy, compared to condensed tannins, to contain the accumulation of indole compounds.

Keywords. Cardus – Tara – Chestnut – Mimosa – Gambier.

Effet des tannins sur la teneur en indoles et la flaveur "pastorale" de la viande d'agneau

Résumé. *Scatols et indoles dérivent de la dégradation du tryptophane dans le tractus digestif et sont responsable des goûts désagréables de la viande et de la graisse d'agneau. Leur formation et leur absorption peuvent être contrôlées à travers les stratégies alimentaires. En particulier, les polyphénols de la diète réduisent la biodégradation du tryptophane et sont censés atténuer le goût "pastoral" de la viande ovine. L'expérimentation visait à évaluer l'effet de la nature des polyphénols sur les caractéristiques sensorielles et chimiques de la viande d'agneau. Cinquante-trois agneaux de 70 jours d'âge ont été répartis en six groupes de 7-10 animaux chacun et ont reçu les diètes expérimentales pendant 75 jours. Le groupe témoin recevait une diète formulée avec de l'orge, du son, de la luzerne déshydratée et de la farine de soja. Les autres groupes recevaient la diète témoin avec les composantes suivantes : i) 15% de cardus, en substitution partielle de la luzerne déshydratée; ii) 4% d'extrait de tannins de tara (tannins hydrolysables – acide gallique), iii) 4% d'extrait de tannins de châtaigne (tannins hydrolysables – acide ellagique), iv) 4% d'extrait de tannins de mimosa (tannins condensés – profisetinidine) et, v) 4% d'extrait de tannins de gambier (tannins condensés – catéchine). La graisse périrénale a été extraite dans du méthanol et passée à travers une colonne Sep-Pak C18 pour analyse du profil en acides gras. La teneur en scatols et indoles a été quantifiée en utilisant un système HPLC avec un détecteur de fluorescence. Il avait été demandé à un panel entraîné de faire l'Analyse Descriptive Quantitative des échantillons de viande ovine provenant du muscle longissimus dorsi ainsi qu'une évaluation olfactive des échantillons de gras. Les diètes riches en tannins hydrolysables réduisent significativement la flaveur "pastorale" de la graisse périrénale, et en même temps elles ont montré une plus grande efficacité, en compaison aux tannins condensés, pour limiter l'accumulation de composés d'indoles.*

Mots-clés. *Cardus – Tara – Châtaignier – Mimosa – Gambier.*

I – Introduction

Lamb meat has a characteristic “pastoral” flavor (Schreurs *et al*., 2008) that is often not appreciated by consumers (Sañudo *et al*., 2003). Recently, there is a growing interest in tannin-rich plants' addition to animal diets because they are claimed to mitigate “pastoral” flavor of lamb meat and fat (Vasta and Luciano, 2011). These bioactive compounds, most of which derive from proteins, can interfere with the rumen microbial ecosystem reducing synthesis of molecules that cause off-flavor of lamb meat. “Pastoral” flavor has been attributed to the presence of specific compounds in the meat, particularly indole and skatole (3-methylindole), branched-chain fatty acids (BCFA), phenolic compounds, sulphides, mercaptans and thiols (Schreus *et al*., 2008). Using forages with high content of endogenous tannins or adding exogenous tannins to diets can mitigate “pastoral” flavor because of the formation of tannin-protein complexes in the rumen that prevent enzymatic hydrolysis of proteins and free amino acids availability (Hagerman, 1992).

Tannins' effect depends on different factors as the nature of the tannin, microbial species with which they interfere and protein aminoacidic composition (Patra and Saxena, 2011).

The aim of this study was to check the effect of adding different tannins to the diet on sensory and chemical characteristics of lamb meat.

II – Material and methods

1. Experimental design, animals and diets

Fifty-three lambs of 70 days of age were divided in six groups of 7-10 animals each and fed the experimental diets for 75 days. The control group received a diet formulated with barley, bran, dehydrated alfalfa and soybean meal. Four of the remaining groups were fed the control diet supplemented with a 4% of one of the following commercial tannin extracts: i) tara (hydrolysable tannins – gallic acid – titer purity 53%), ii) chestnut (hydrolysable tannins – ellagic acid – titer purity 75%), iii) mimosa (condensed tannins-profisetinidin – titer purity 65%) and, iv) gambier (condensed tannins- catechin – titer purity 48%). The remaining group received the control diet with 15% cardus in partial substitution of dehydrated alfalfa

2. Sensory panel evaluation

Ten trained panelists, ranging in age between 22 and 55 years, carried out the Quantitative Descriptive Analysis (QDA) of sheep meat samples from *longissimus dorsi* muscle as well as an olfactory evaluation of fat samples. The preliminary sessions were performed to develop a common vocabulary that allowed assessors to use the same terms to describe their perceptions. After that, assessors carried out the sensory evaluation in four replicas, each divided in two sessions. Assessors evaluated 26 descriptors for *longissimus* muscle samples, and six for fat olfactory evaluation. Panellists assigned to each sample and for each sensory attribute a score between 1 (absence of the sensation) and 10 (sensation extremely intense) using a linear scale. Results were collected using software FIZZ v.2.47B (Biosystemes, Couternon, France).

3. Indoles analysis in perirenal fat

Skatole and indole content was measured according to the method of Tuomola et al. (1996). A sample of 2,5 g of fat was homogenized in 10 ml of methanol together with 30 µl of 2-methylindole as internal standard by means of an Ultra Turrax. Homogenate was cooled for 30 min at -20°C and then centrifuged at 4000 rcf for 10 min. The supernatant was filtered through a Sep-Pak C18 column and 2 ml of eluate injected in an HPLC system (Schimadzu LC-20AT-Germany) with a fluorescence detector.

4. Statistical analysis

ANOVA and multivariate analysis (Principal Components Analysis) of sensory data were performed using the statistical software PanelCheck (Nofima Mat & DTU -Informatics and Mathematical Modelling; Norway). The evaluation of the effect of tannins nature on individual descriptors considered both product and assessor effect, assuming the latter as a random factor. ANOVA of chemical data and their correlation with sensory data were carried out using the statistical software SPSS ver.17 (SPSS Inc., Illinois).

III – Results and discussion

Results obtained from sensory evaluation are summarized in Figure 1.

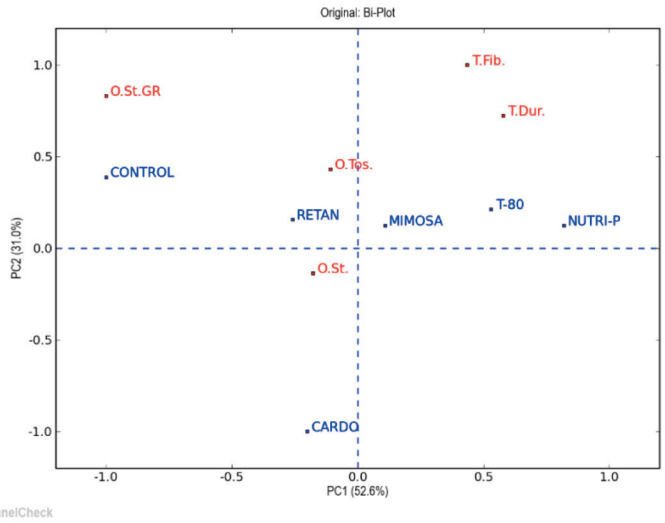

Fig. 1. Map of diets (Control; RETAN = Gambier; Mimosa; Cardus; T-80 = Tara; NUTRI-P = Chestnut) and significant attributes (O.St. GR = fat pastoral flavor; O.St.=meat pastoral flavor; O.Tos. = toasted odor; T.Fib. = meat fibrosity; T.Dur = meat hardness) derived from Principal Components Analysis of lamb meat and fat sensory profiles.

The first two components of the Principal Components Analysis explained 83% of the variability between samples. Except for the diet including cardus, all the others were lined up along the first component, highly related to “pastoral” flavor. Perirenal fat of animals supplemented with chestnut and tara tannins was different from the others because of a lower score related to “pastoral” flavor. The diet including cardus was different from the others because it was lined up along the second component, mainly related to the texture of the meat.

Differences between diets including tannins (excluding control diet and that including cardus) are shown in Fig. 2. Diets including hydrolysable tannins (tara and chestnut) were in opposite quadrants than those including condensed tannins (mimosa and gambier). Along the second component, the diet including mimosa tannins was different than the diet including gambiertannins due to a higher correlation with rancid odor, and a lower correlation with “pastoral” flavor. In the other side, the diet including chestnut tannins was different than that including tara tannins because of a higher correlation with sweet fat odor as well as a lower correlation with meat toasted odor.

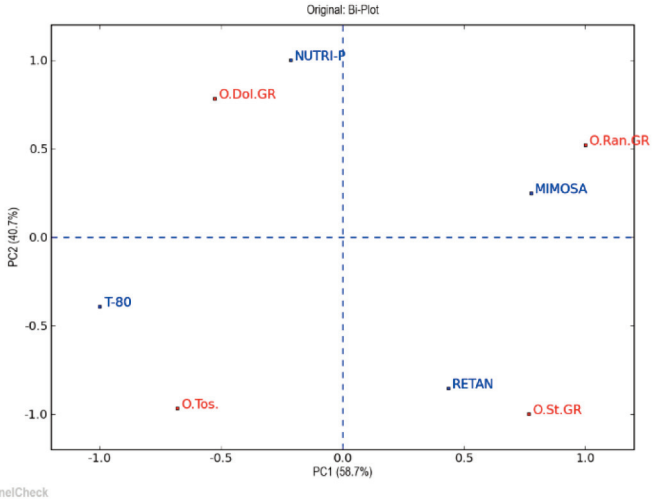

Fig. 2. Map of diets (RETAN = Gambier; Mimosa; T-80 = Tara; NUTRI-P = Chestnut) including tannins, and significant attributes (O.Ran.GR = fat rancid odor; O.Dol.GR. = fat sweet odor; O.St.GR = fat pastoral flavor; O.Tos. = toasted odor) derived from Principal Components Analysis of lamb meat and fat sensory profiles.

Chemical analysis aimed to assess the implication of skatole and indole in the perception of pastoral flavor, and the results are reported in Table 1. Perirenal fat of lambs fed diets including hydrolysable tannins (chestnut and tara) had a lower indole content than that from animals fed diets including condensed tannins (mimosa and gambier).

Table 1. Indole, skatole and total indoles content (ng/g) in lamb perirenal fat

	Control	T-80 (Tara)	Nutri-P (Chestnut)	Mimosa	RETAN (Gambier)	Cardus	DSE	*P*
Indole	25.4[a]	14.1[b]	15.1[b]	27.4[a]	32.0[a]	27.9[a]	2.19	.015
Skatole	9.6	10.4	10.7	11.9	4.7	16.7	2.14	.545
Indoles	35.0	24.5	25.7	39.3	36.8	44.5	3.40	.380

These results are related to "pastoral" flavor score of perirenal fat (Fig. 3). Diets including hydrolysable tannins had a significantly reduced "pastoral" flavor, whereas hydrolysable tannins demonstrated a greater efficacy than condensed tannins in containing indoles' tissue accumulation, particularly indole.

As reported by Peterson and Reineccius (2003), indole and skatole can confer to animal products floreal and sweet flavor at low concentration, whereas flavor becomes undesirable at high concentration (Young *et al.,* 2003).

Most of the studies reported in the literature consider only plants' endogenous condensed tannins, which complex structure allows them to be more resistant to ruminal degradation (Piluzza *et al.,* 2014). The results of this research, that compares the use of different tannins, are probably due to different factors that could have modify rumen degradation such as the exogenous origin of tannins and their different purity (from 48% in gambier tannins to 75% in chestnut tannins). All these factors could have changed the relationship between polyphenols availability and the the rate of degradation of proteins in the rumen.

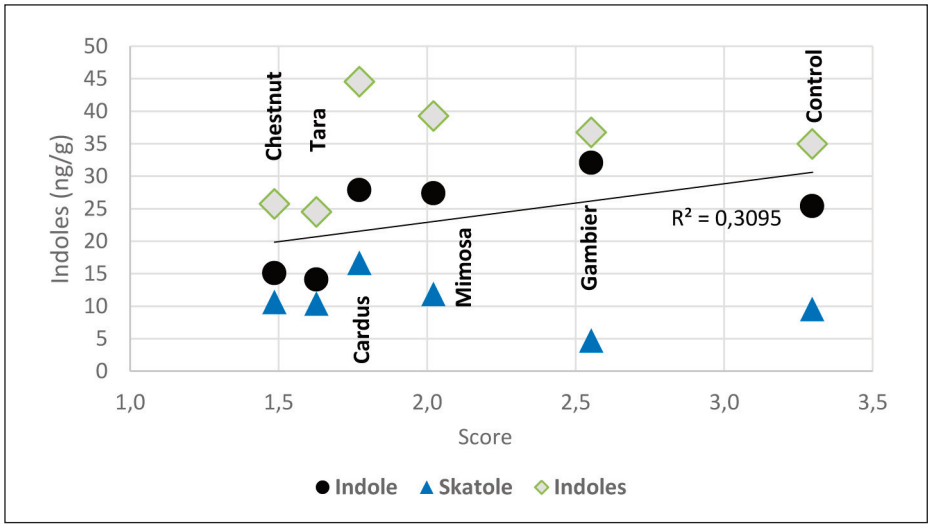

Fig. 3. Relation between "pastoral" flavor (Score) and indoles content in lamb perirenal fat.

IV – Conclusions

Hydrolysable tannins, even though at a low level of inclusion (4%) in the diet, can modify sensory characteristics of lamb meat and fat, particularly reducing "pastoral" flavor. This effect is related to a low content of indoles in the animals' fat.

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