Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

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To cite this article: Q. Priolo & V. Vasta (2007) Effects of tannin-containing diets on small ruminant meat quality, Italian Journal of Animal Science, 6:sup1, 527-530

To link to this article: https://doi.org/10.4081/ijas.2007.1s.527

MAIN LECTURE

Effects of tannin-containing diets on small ruminant meat quality

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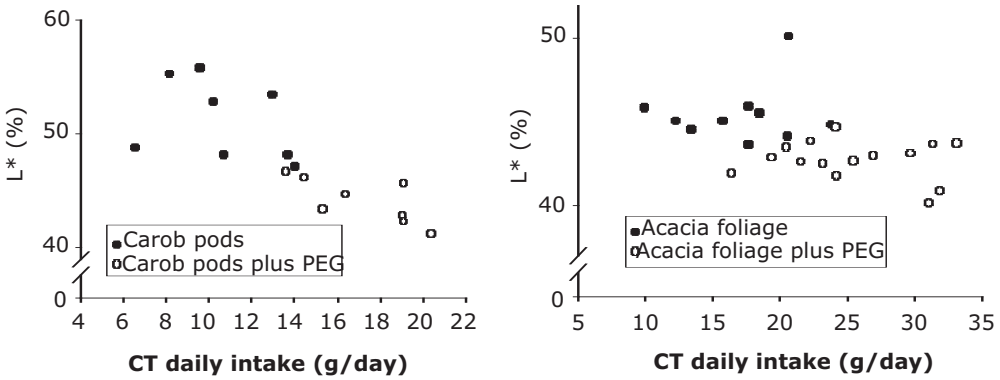
ABSTRACT: Tannins are phenolic compounds present in several forages, tree leaves and by-products used for small ruminant feeding in the Mediterranean area. Although the effects of dietary tannins on small ruminant growth performances have been largely studied, only in the last ten years researchers have started to study the effects of tannins on meat quality. Meat from small ruminants given tanniniferous diets is lighter in colour compared to meat from animals given the same diets but in which the effects of tannins have been eliminated by the supplementation of polyethylene glycol. This result has been obtained with tannins from different feeds (carob pulp, acacia leaves or sulla fresh herbage). Recent *in vitro* studies seem to indicate that a possible effect of tannins on meat colour could be due to a reduced microbial biosynthesis of vitamin B12 which is a precursor for the synthesis of haeme pigments. Meat from lambs given carob contains less conjugated linoleic acid compared to meat from animals fed the same diet but supplemented with polyethylene glycol. A possible explanation of this result has been proposed recently after an *in vitro* experiment on linoleic acid isomerase; it has been shown that when cattle ruminal fluid is incubated with tannins exctracted from acacia, carob or quebracho, the conjugated linoleic acid formed from linoleic acid was lower compared to the mount of conjugated linoleic acid produced in tannin-free rumen fluid. Also, the ruminal biosynthesis of odour-active compounds seems to be affected by tanniniferous diets. This is the case of skatole (3-methylindole) and indole which confer unpleasant flavour connotations to lamb meat.

INTRODUCTION – Feeding costs are one of the major problems in the economic balance of small ruminant farmers. In several Mediterranean areas animal feed production is difficult and farmers purchase expensive concentrates. Many shrubs and by-products have been studied in these years by researchers from the three Mediterranean continents with the aim to replace expensive conventional feeds (Silanikove *et al.*, 1994; Ben Salem *et al.*, 1996; Priolo *et al.*, 1998). In many cases a problem with the use of alternative feeds is the presence of anti-nutritional factors such as tannins, phenolic compounds found in many forage plants, particularly in a variety of legume forages (Sarkar *et al.*, 1976; Terrill *et al.*, 1992) and tree leaves (Kumar and Vaithiyanathan, 1990; Silanikove *et al.*, 1994). Tannins are widespread in tropical trees, shrubs and herbaceous plants (Kumar and Vaithiyanathan, 1990; Rittner and Reed, 1992; Jackson *et al.*, 1996) and tannin-containing feeds have been largely used as replacement or in association to conventional feeds. Negative effects exerted by dietary tannins include reduced absorption of some minerals (Waghorn *et al.*, 1994a), reduction of protein digestibility and amino acid (AA) absorption (Waghorn *et al.*, 1994b), reduction of rumen protein utilisation (Jones and Mangan, 1977; Barry and Duncan, 1984), and of voluntary intake (Reed, 1995), reduction of microbial activity in the rumen (Nuñez-Hernandez *et al.*, 1991) and toxic effects reflected by damage of kidney and liver (Kumar and Singh, 1984). In the rumen, tannins bind with plant proteins, reducing their availability for microbial growth. As a consequence of that, the rate and extent of fibre digestion is also reduced with consequent reduction of voluntary intake, metabolizable energy availability and AA absorption. Tannins react preferentially with polyethylene glycol (PEG) and the supplementation of PEG has been largely used to eliminate and to evaluate the effects of tannins (Decandia *et al.*, 2000). Only in the last few years, a number of studies have been focused on the effects of dietary tannins on small ruminants meat quality. These studies are here reviewed.

EFFECTS OF TANNINS ON MEAT COLOUR – In a trial aimed to compare two sorghum varieties with different content of tannins, lambs fed the strain containing the higher level of tannins showed a meat lighter in colour (Verna *et al.*, 1989). Later, Priolo *et al.* (1998) found that feeding tannins from carob pulp in partial replacement of barley to

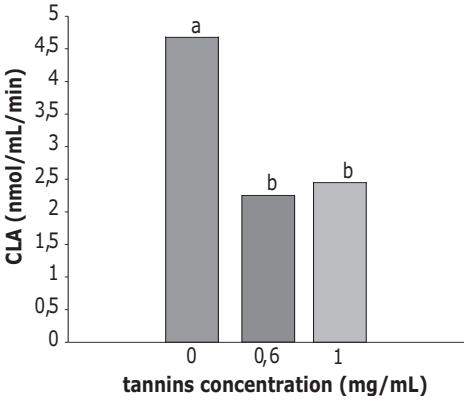
Figure 1. Effect of dietary tannins on lamb meat lightness (L*). fulfilled circles refer to animals fed a diet containing tannins; empty circles refer to animals receiving a diet containing tannins plus PEG (from Vasta *et al.*, 2007a).



Comisana lambs, increased *longissimus* muscle (1 hr blooming at 4°C) lightness (L*). Animal growth rate, carcass weight, carcass fatness and muscle ultimate-pH were comparable between groups in this experiment. The authors hypothesised that tannins were responsible for the differences found in meat colour. A second experiment designed to evaluate the specific effect of carob tannins on lamb growth and meat quality (Priolo *et al.*, 2000), showed that when the effects of tannins from carob pulp are eliminated by PEG supply, Comisana lamb *longissimus* muscle was significantly darker (lower L*). However in this experiment the growth rate between animals was very different due to the high astringency of tannins. Another experiment (Priolo *et al.*, 2002) evaluated the effect of tannins from *Acacia cyanophylla* foliage on meat quality of Barbarine male lambs. The *longissimus* muscle (2 hr blooming at 4°C) of animal that did not receive PEG was significantly lighter compared to that of the supplemented animals. Also, in a third experiment in which Comisana lambs were fed sulla (*Hedysarum coronarium*) with or without PEG, the meat of the animals not receiving PEG was lighter in colour compared to the supplemented lambs (Priolo *et al.*, 2005). This result, together with those of Priolo *et al.* (2000 and 2002) indicates that tannins from different plant species have similar effects on lamb meat colour. The effect of tannins from acacia and carob on lamb meat colour is showed in Figure 1.
Zembayashy *et al.* (1999) reported that feeding tea leaves to Japanese heifers reduced muscle iron content. It was found also a strongly negative correlation between muscle iron and meat lightness, and it is concluded that feeding tea leaves would be effective in increasing the lightness of the meat. Tea leaves are rich in catechins as reported by the authors and the two findings appear to be correlated. The mechanism of action of tannins (or catechins) on meat colour is not clear. Tannins seem not to influence iron absorption in ruminant: Waghorn *et al.* (1994a) found no difference in Fe absorption in 12 months old Romney wethers grazing on *Lotus pedunculatus* with or without PEG. In an experiment with cattle fed tannins from oak (*Quercus incana*) leaves, Garg *et al.* (1992) found that although the blood haemoglobin was affected by tannin poisoning, no differences in blood iron were present. Similar results have been obtained by Priolo *et al.* (2000) with tannins from carob pulp. It is therefore likely that the tannins do not affect ruminant Fe absorption but hamper the successive utilisation of the iron for synthesis of haemoglobin as suggested by Garg *et al.* (1992). In a recent *in vitro* study on cattle ruminal fluid, it was found that tannins extracted from carob pulp, from acacia leaves or from quebracho reduced the microbial biosynthesis of vitamin B12 (Vasta V., Makkar H.P.S., Priolo A., unpublished data), which is a precursor for the synthesis of haemoglobin. According to these findings, it is likely that a reduced biosynthesis of haemoglobin caused by dietary tannins could result in a lighter colour in meat.

EFFECTS OF TANNINS ON RUMINAL BIOHYDROGENATION AND INTRAMUSCULAR FATTY ACID COMPOSITION – Only few studies have investigated on the effects of feeding tanniniferous feed on lamb meat fatty acid composition with a particular focus on *cis*-9 *trans*-11 conjugated linoleic acid (CLA), a fatty acid which has favorable effects on human health. It is well known that CLA is partially synthesized by ruminal microorganism during the biohydrogenation of dietary polyunsaturated fatty acids (PUFA). Priolo *et al.*

Figure 2. The effect of different concentrations of tannins (0, 0.6 or 1.0 mg/mL of incubation medium) extracted from acacia, carob or quebracho on the in vitro production of CLA by linoleic acid isomerase from cattle ruminal fluid (Vasta V., Makkar H.P.S., Mele, M. and Priolo A., unpublished results).



(2005) hypothesized that tannin-rich diets could reduce the accumulation of CLA in lamb meat through an impairment of the activity of ruminal microorganisms. However, in a first study, when Comisana lambs were fed sulla, the supplementation of polyethylene glycol did not affect fatty acid composition in *longissimus* muscle, probably because of the low amount of tannins (1.8% on DM) in sulla (Priolo *et al.*, 2005).On the contrary, Vasta *et al.* (2007b) found that feeding lambs a diet containing tannins (2.7% on DM) from carob reduced the accumulation of CLA and of *trans*-vaccenic acid (*trans*-11 C18:1, TVA; the last intermediate of ruminal biohydrogenation) in intramuscular fat, compared to animals receiving the same diet but supplemented with PEG. Recently, an *in vitro* experiment on linoleic acid isomerase (Vasta V., Makkar H.P.S., Mele M., Priolo A., unpublished results) has shown that when cattle ruminal fluid was incubated with tannins extracted from acacia, or from carob or from quebracho, the CLA formed from linoleic acid was lower compared to the amount of CLA produced in tannin-free rumen fluid (Figure 2). Khiaosa-Ard *et al.* (2007) have found that when cow ruminal fluid was incubated *in vitro* for 20 days with grass hay plus tannins extracted from *Yucca schidigera*, the amount of *trans*-vaccenic acid produced in the fermentor was two-fold compared to the tannin-free system, while CLA was not affected by the presence of tannins. Probably, tannins have reduced or inhibited the biohydrogenation of *trans*-vaccenic acid to stearic acid, resulting in the accumulation of TVA. In the muscle and in the mammary gland TVA can be converted to CLA by the enzyme Δ-9 desaturase (Bauman *et al.*, 1999). Further studies are needed to better understand how dietary tannins can influence ruminal metabolism and therefore products fatty acid composition.

EFFECTS OF TANNINS ON MEAT ODOUR-ACTIVE COMPOUNDS – Among the aroma-active compounds skatole (3-methyl indole) and indole negatively affect meat flavour, being associated with a faecal odour (Young and Baumeister, 1999). Skatole is synthesized by ruminal microorganisms form the decarboxylation and deamination of the aminoacid tryptophan, and its accumulation in lambs fat is enhanced by feeding green herbages (for a review see Vasta and Priolo, 2006). Priolo *et al.* (2005) reported that when Comisana lambs were fed a tanniniferous herbage (sulla) the deposition of skatole in perirenal fat was not affected by the supplementation of PEG, and also was higher compared to a group of lambs fed a maize-based diet. On the contrary, Tavendale *et al.* (2005) have shown in an *in vitro* experiment that tannins from *Dorycnium rectum* reduced the biosynthesis of skatole into sheep rumen inocula. Also, Schreurs *et al.* (in press) have found that supplementing grape seed extract (GSE, a tannin containing by-product) to lambs fed green forages reduced the concentration of skatole and indole in plasma and rumen fluid, but not into animal fat, compared to lambs not receiving GSE. These results suggest that dietary tannins can affect the biosynthesis of skatole and indole and further investigations are needed to understand if dosing tannins to the animals can improve meat flavour.

CONCLUSION – Dietary tannins affect meat quality in several aspects. Meat from animals fed tanniniferus diets appear to be lighter in colour compared to meat from animals fed the same diets but supplemented with polyethylene glycol. A possible explanation of this result is the reduced production of B12 vitamin by ruminal microorganisms, as found *in vitro* resulting in a reduced production of heamoglobin. *In vitro* studies have also demonstrated that tannins reduce ruminal biohydrogenation, affecting the biosynthesis of conjugated linoleic acid and of its precursor, trans-vaccenic acid. However, *in vivo* studies have reported converse results about the effect of feeding tannins and the accumulation of CLA in lamb meat and further researches are needed to deepen the knowledge on this topic. Dietary tannins can also reduce the production by ruminal microorganisms of some volatile compounds such as skatole and indole and this can have important implication for ameliorating meat flavour.

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