

The effect of supplementing chestnut tannins on the productive results of finishing lambs fed rations deficient in metabolizable protein

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Summary

The aim of this study was to investigate the effects of chestnut tannins in metabolizable protein deficiency, on dry matter (DM) intake, growth performance of finishing lambs, feed efficiency and digestibility of nutrients. The study included 30 crossbred lambs, blocked by body weight (BW) and divided into three groups, depending on the concentrations of added chestnut tannins in the rations (0, 10 and 20 g/kg DM for CON, 10T and 20T group, respectively). Rations were determined by BW and expected average daily gain (ADG), with a deficiency in metabolizable protein of about 15%. All groups received the same amount of daily feed. Dry matter intake, ADG and indicators of efficiency such as the Kleiber ratio (KR) and protein efficiency ratio (PER) were estimated. The digestibility of the rations was determined in three collection periods. Dry matter intake did not differ among groups. Growth performances for the whole experiment were significantly better for the 20T group than CON ($p < 0.05$), while no differences were established for group 10T. Group 20T achieved the highest ADG and total gain ($228 \text{ g/day} \pm 27.8$; $13.7 \text{ kg} \pm 1.7$), with the best average conversion of dry matter (4.4 kg DM/kg BW), KR (15.6), and PER (1.6). In all three collections, dry matter and crude protein digestibility was higher for CON, while the digestibility of ether extract was higher for the 20T group than CON. Based on these results, it can be concluded that a ration of chestnut tannins in the concentration of 20 g/kg DM, in conditions of protein deficiency, can have a positive effect on lamb performances. This can be partially explained by the ability of tannins to form complexes with proteins, which can change intestinal protein flow and utilization.

Keywords: Growth, polyphenols, feed efficiency, digestibility

Zusammenfassung

Die Wirkung der Ergänzung von Kastanien-Tanninen auf die Produktionsergebnisse von Lämmern bei Proteinmangelrationen

Das Ziel dieser Studie war, die Wirkungen von Kastanien-Tanninen in Rationen mit unzureichender Versorgung mit metabolisierbarem Protein auf die Aufnahme von Trockenmasse (DM), die Wachstumsleistung von Mastlämmern, den Futteraufwand und

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die Verdaulichkeit von Nährstoffen zu untersuchen. Die Studie umfasste 30 Kreuzungslämmer, die durch das Körpergewicht (BW) und die Konzentrationen von zugesetzten Kastanien-Tanninen in der Nahrung (0, 10 und 20 g/kg DM für die Kontrolle, 10T- bzw. 20T-Gruppe) in drei Gruppen unterteilt wurden. Die Rationen wurden durch das Körpergewicht (BW) und die erwartete durchschnittliche tägliche Zunahme (ADG) mit einem Mangel an metabolisierbarem Protein von etwa 15% definiert. Alle Gruppen erhielten die gleichen Futtermengen. Die DM-Aufnahmen, ADG-Werte, und Verwertungsgrößen wie das Kleiber-Verhältnis (KR) und das Protein-Effizienz-Verhältnis (PER) wurden geschätzt. Die scheinbare Verdaulichkeit wurde in drei Sammelperioden ermittelt. Die DM-Aufnahme unterschied sich nicht zwischen den Gruppen. Die Wachstumsleistungen für das gesamte Experiment waren für die 20T-Gruppe signifikant besser als für die Kontrollgruppe ($p < 0,05$), während für die 10T-Gruppe keine Unterschiede festgestellt wurden. Gruppe 20T erreichte den höchsten ADG und Gesamtzuwachs ($228 \text{ g/Tag} \pm 27,8$; $13,7 \text{ kg} \pm 1,7$) mit der besten durchschnittlichen Umwandlung von Trockenmasse ($4,4 \text{ kg DM/kg BW}$) und den günstigsten Werten für KR (15,6) und PER (1,6). In allen drei Sammelperioden war die Verdaulichkeit von DM und Rohprotein bei der Kontrolle höher, während die Verdaulichkeit des Rohfetts bei der 20T-Gruppe höher war als bei der Kontrolle. Aus den Ergebnissen kann geschlossen werden, dass Kastanientannine in einer Konzentration von 20 g/kg DM Futter bei Proteinmangel einen positiven Effekt auf die Leistung von Mastlämmern haben können. Dies kann teilweise durch die Fähigkeit von Tanninen erklärt werden, Komplexe mit Proteinen zu bilden, die den Proteinfluss durch den Verdauungstrakt und seine Verwertung verändern können.

Schlüsselwörter: Wachstum, Polyphenole, Futteraufwand, Verdaulichkeit

1 Introduction

Tannins are a heterogeneous group of water-soluble polyphenolic polymers with a relatively high molecular weight, usually divided into hydrolysable (HT) and condensed tannins (CT) (PATRA and SAXENA, 2011). They are defined as water-soluble polyphenolic substances that have the ability to form soluble or insoluble complexes with proteins (HASSANPOUR et al., 2011), but they can also form complexes with polysaccharides, nucleic acids, steroids and other biomolecules (CHAICHISEMSARI et al., 2011). Tannins and proteins form stable complexes at pH 6.0 – 7.0 (as in rumen), but at highly acidic (as in abomasum pH < 3.5) or highly alkaline mediums (> 8.5 as in small intestine) these complexes dissociate. The use of tannins can lead to more optimal protein utilization by affecting rumen fermentation and biohydrogenation (SARNATARO and SPANGHERO, 2020), it can affect dry matter (DM) intake (KRUEGER et al., 2010; DE S. COSTA et al., 2020), digestibility (DEAVILLE et al., 2010), and further lead to improved animal productivity (higher growth (RIVERA-MENDEZ et al., 2017), more favorable diet conversion (MEZZOMO et al., 2016), higher yields as well as changes in the quality of meat (MAHACHI et al., 2020) and milk (ALI et al., 2017)). On the other hand, if dietary concentrations of tannins are too high, negative effects can be manifested, such as: low intake due to an unpleasant (astringent) taste (VALENTI et al., 2020), reduced digestibility of DM and organic matter (OM) (DEAVILLE et al., 2010), lower growth rate (DE S. COSTA et al., 2020), various forms of animal intoxication (JERONIMO et al., 2016), etc. In recent years, tannin extracts obtained from wood species, primarily chestnut (*Castanea sp.*), oak (*Quercus sp.*), mimosa (*Acacia mearnsii*), tara (*Cesalpinia spinosa*) and quebracho (*Schinopsis sp.*) have been increasingly used in the diet of ruminants. Chestnut tannins contain large concentrations of HT (COMANDINI et al., 2014). The aim of this study is to investigate the effect of different levels of chestnut tannins, a source of HT, on dry matter intake, growth performance, feed efficiency

and apparent digestibility in finishing lambs fed on rations deficient in metabolizable protein (MP), concerning the hypothesis that tannins can affect better protein utilization.

2 Materials and methods

The experiment was conducted at the Institute of Animal Husbandry in Belgrade, Serbia, in strict accordance with standard ethical norms, and was approved by the Veterinary Administration, the Ministry of Agriculture, Forestry and Water Management (Permit number 323-07-04346/2019-05).

2.1 Animals, housing, treatments and rations

The study included 30 crossbred lambs (local breed Pirot Pramenka × Merinolandschaf × Ile de France), introduced into the experiment after weaning (at the age of 60 ± 5 days), and blocked by body weight (BW); three treatment groups were formed with 10 lambs (5 females and 5 males) in each group. During the experiment, the lambs were housed in a closed shed, within grouped pens that included wheat straw deep mat, and had access to water *ad libitum*. The experiment lasted 66 days, including 6 days for adaptation and 60 days for data collection.

The rations were formulated based on BW and the expected average daily weight gain (ADG) of 200–300 grams, following recommendations of the Dutch CVB for sheep nutrition (CVB, 2018). Three rations were formed, and given to lambs over the course of 20 days. These rations were calculated to be deficient at 15% of MP requirements, and fed under the assumption that tannins will positively affect their MP supply. Within each ration, three treatments were defined, and their structure, composition and nutritive value are shown in Table 1. The nutrient requirements for lambs are shown in Table 2.

The treatments consisted of one ration without chestnut tannins (control – CON), and two rations with 10 (10T) and 20 g (20T) tannins/kg dry matter (DM). Farmatan Plus® (Tanin Sevnica d.d., Slovenia) was used as a source of tannins. The product, according to the manufacturer's specification, was obtained by aqueous extraction and consisted of $73 \pm 2\%$ chestnut extract (*Castanea sativa* Mill.) and 0.1% sweetener (stevia extract). To achieve the mentioned concentrations of chestnut tannins in rations, the fact that Farmatan Plus® contains an average of 55% tannins (EFSA, 2005) was taken into consideration. The chemical composition of Farmatan Plus® is shown in Table 3. This chestnut product was mixed in concentrated part of the rations. Nine rations were produced (three for each period and each treatment, for experimental days 1–20, 21–40 and 41–60, respectively).

2.2 Intake, growth performance and feed efficiency

The average BW at the beginning of the experiment for CON, 10T and 20T was $20.5 \text{ kg} \pm 2.2$ (\pm standard deviation – SD), $20.5 \text{ kg} \pm 2.4$ and $20.5 \text{ kg} \pm 2.1 \text{ kg}$, respectively.

A daily feed intake was estimated for every group (from day 1 until day 60), based on the difference between the feed offered and feed refusals next day. All groups received the same amount of daily feed, which was determined by the group that had the lowest intake on the previous day, so that the group with the lowest intake had a daily feed refusal of 10%, with respect to the roughage and concentrated feed ratio.

The change in BW was controlled at 10 day intervals, so that during the use of one ration, the BW was measured twice. The BW of lambs was measured by using a small animal scale, with an accuracy of 0.10 kg. The average daily gain (ADG) was determined for every animal by dividing the total weight gain with the number of days in the experimental period.

Tab. 1. The structure, chemical composition and nutritive value of the rations
Struktur, chemische Zusammensetzung und Nährwert der Rationen

Rations Feeds/Group	Ration 1			Ration 2			Ration 3		
	CON	10T	20T	CON	10T	20T	CON	10T	20T
Meadow hay, g/kg	357.9	351.5	345.0	476.5	468.2	460.0	519.7	511.3	502.9
Alfalfa hay, g/kg	175.1	172.00	168.9	/	/	/	/	/	/
Corn silage, g/kg	/	/	/	63.5	62.4	61.3	173.2	170.4	167.6
Corn, grain, g/kg	251.3	246.8	242.3	190.6	187.3	184.0	92.0	90.6	89.1
Sunflower meal, g/kg	83.8	82.3	80.8	146.1	143.6	141.0	157.09	154.5	151.9
Wheat bran, g/kg	114.2	112	110.1	95.3	93.6	92.0	37.9	37.3	36.7
Salt, g/kg	4.2	4.0	4.0	1.9	1.9	1.8	3.9	3.8	3.8
Monocalc. ph, g/kg	1.5	1.5	1.5	14.0	13.7	13.5	8.7	8.5	8.4
Premix, g/kg	12.0	11.7	11.5	12.1	11.9	11.7	7.6	7.5	7.3
Farmatan, g/kg	0.0	18.0	35.9	0.00	17.4	34.7	0.00	16.1	32.3
Chemical composition of ratio, g/kg DM*									
Dry matter, g/kg feed	864.3	868.7	874.9	816.7	817.7	820.1	752.5	753.0	759.7
Crude protein	145.1	140.6	146.5	132.2	132.1	130.6	123.2	124.7	120.0
Ether extract	19.4	18.6	18.9	18.3	16.9	17.4	13.7	13.5	13.7
Crude fiber	162.8	164.4	169.8	175.5	176.9	164.7	194.1	195.8	190.9
Ash	95.5	104.8	95.9	95.5	96.9	94.9	89.1	90.1	90.0
NDF	406.6	400.5	401.6	438.9	438.3	418.8	463.4	466.7	457.0
ADF	254.9	254.7	263.1	281.0	278.2	266.8	306.2	305.7	299.3
NFC	333.5	335.6	337.2	315.1	315.8	338.4	310.6	305.1	319.3
Ca	8.8	12.2	8.8	10.0	13.7	12.6	8.0	9.4	9.8
P	5.0	4.8	5.3	5.2	5.7	4.7	4.6	5.1	5.2
PeHCL	10.8	11.1	10.8	14.1	14.1	13.9	15.5	15.5	15.0
Reducing sugars	44.8	43.9	47.4	45.6	46.6	46.4	41.1	42.0	42.6
Total polyphenols**	72.2	76.3	113.4	66.1	87.7	139.6	81.4	82.8	124.6
Flavonoids**	28.9	35.8	50.3	39.7	58.9	69.3	46.3	54.1	62.2
Cond. tannins**	5.3	6.3	6.8	6.4	7.0	7.1	6.3	6.9	7.0

Tab. 1. Continued
Fortsetzung

Rations Feeds/Group	Ration 1			Ration 2			Ration 3		
	CON	10T	20T	CON	10T	20T	CON	10T	20T
Nutritive value of rations according to CVB 2016***									
NE _{meat} , MJ/kg DM	5.7	5.6	5.6	5.5	5.4	5.3	5.2	5.1	5.0
MP, g/kg DM	69.5	68.3	68.1	60.8	59.7	58.7	54.2	53.3	52.4
bRp	0.1	0.1	0.0	0.6	0.6	0.6	0.2	0.2	0.2

Vitamin-mineral premix contained (per kg) 500,000 IU vit A; 50,000 IU vit D₃; 4,000 mg vit E; 24% Ca; 4% P; 5% Na; 2% Mg; 1,700 mg Zn; 1,200 mg Mn; 1,350 mg Fe; 300 mg Cu; 4 mg Co; 4 mg Se; 26 mg I

* NDF – neutral detergent fibre; ADF – acid detergent fiber; NFC – non-fibrous carbohydrates; Ca – calcium; P – phosphorous; PeHCL – ash in hydrochloric acid.

** Total polyphenols expressed as g GAE/kg DM – grams equivalent gallic acid in kg dry matter; Flavonoids and condensed tannins expressed as g CAE/kg DM – grams equivalent catechin in kg dry matter.

*** N_{meat} – Net energy, expressed in MJ/kg DM; MP – metabolizable protein, expressed as g/kg DM; bRp – Rumen degraded protein balance.

Tab. 2. Nutrient requirements for lambs, with expected average daily gains of 200–300 g, depending on body weight, according to CVB (2018)
Nährstoffbedarf von Lämmern mit erwarteten durchschnittlichen Tageszunahmen von 200–300 g, abhängig vom Körpergewicht, gemäß CVB (2018)

Parameter/Body weight (kg)	25	30	35
Dry matter intake, kg/day	0.6 – 1.1	0.8 – 1.3	1.0 – 1.5
NE _{meat} , MJ/kg DM/day	5.5 – 7.8	6.4 – 8.9	7.1 – 10.0
Metabolizable protein, g/kg DM/day	79 – 107	83 – 113	86 – 119
Rumen degraded protein balance	≥ 0.00	≥ 0.00	≥ 0.00

Based on BW and feed intake changes, feed efficiency was estimated by calculating the feed conversion (FC), expressed in kg DM/kg gain, Kleiber ratio (KR) and protein efficiency ratio (PER). Feed conversion was calculated by dividing ADG with nutrient intake. The Kleiber ratio was calculated by dividing ADG with metabolic body size (kg^{0.75}), and PER was calculated by dividing ADG with the amount of crude protein (CP) that had been consumed.

2.3 Digestibility assay

Digestibility was estimated with an internal marker and performed in three collection periods, starting on days 14, 29 and 44 of the experiment, which matches with rations 1, 2

Tab. 3. Chemical composition of Farmatan Plus®
Chemische Zusammensetzung von Farmatan Plus®

Parameter	Value
Dry matter (DM), %	89.8
Crude protein, g/kg DM	13.8
Ether extract, g/kg DM	1.2
Crude fiber, g/kg DM	0.5
Ash, g/kg DM	19.4
Total polyphenols, g GAE/kg DM*	361.4
Flavonoids, g CAE/kg DM*	170.1
Condensed tannins, g CAE/kg DM*	13.4

* g GAE/kg DM – grams gallic acid equivalent in 1 kg dry matter; g CAE/kg DM – grams catechin equivalent in kg dry matter.

and 3, respectively. Ash insoluble in hydrochloric acid was an internal marker. Each collection period lasted 6 days. Cotton cloth fecal bags were attached to six animals from each group. Before the start of each collection period and every day of the collection period, using a safe adhesive, new, clean bags were attached to a wool and onto the back of the animal covering the anus. Each day during the collection period, about 100 g of feces was taken from each test animal, and stored in plastic bags. At the end of the collection period, a homogenized fecal sample was made for each animal in the test, which, after determining the CP, was packed in plastic bags and stored in a freezer at -20°C for further analysis.

Digestibility was determined using the following formula:

$$\text{Digestibility\%} = 100 - [(\% \text{ of indicator in diet}) / (\% \text{ of indicator in feces}) \times (\% \text{ of nutrient in feces}) / (\% \text{ of nutrient in diet})] \times 100$$

Based on the above formula, digestibility was determined for the following variables: DM, OM, CP, ether extract (EE), crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF).

2.4 Chemical analysis

The chemical composition was determined on the basis of chemical analyses, while the nutritive value of the rations was estimated by relying on the Dutch CVB recommendations for sheep nutrition (CVB, 2018).

In the feeds and Farmatan Plus®, the concentration of total phenolic compounds was determined spectrophotometrically using the Folin-Ciocalteu method, the content of flavonoids according to MAKAR (2000), and the content of condensed tannins using the vanillin method (NAKAMURA et al., 2003).

After grinding and sieving the samples (feed and feces), the following analyses were performed using the AOAC (2000) methods: DM (method 967.03), ash (method 942.05), CF (method 978.10), EE (method 920.39), ADF (method 973.18), while NDF by method described by VAN SOEST et al. (1991). Crude protein was determined using the Kjeldahl method (ISO, 2005). The content of ash insoluble in hydrochloric acid was determined by combusting organic matter, and then boiled in a 10% hydrochloric acid so-

lution. Nonfiber carbohydrates (NFC) were calculated as $\%NFC = 100 - (\%CP + \%NDF + \%EE + \%ash)$.

2.5 Statistical analysis

The experiment was set up as a one-factor trial with three treatments. The basic statistical indicators included the arithmetic mean and standard deviation. The statistical significance of the differences between all examined parameters was determined using the one way ANOVA, followed by the Least Significant Difference test (LSD). Statistical analysis was performed using the statistical package Statsoft Statistica 7 (STATSOFT, 2003).

3 Results

At the beginning of the experiment, one lamb from CON group was excluded from further analysis for reasons not related to the experimental setup.

Table 4 shows the group intake of DM, CP and energy, while conversions, KR and PER for rations and the whole experiment are shown in Table 5. Intake data were expressed as group mean values, and ADG data were calculated individually, for each animal. Feed intake was measured for all groups, and their daily intake of DM, CP and energy were similar. In first ration, FC, CP conversion and energy conversion were lowest (best), while KR and PER were highest for 20T. In second ration, FC, CP and energy conversion were better, and KR and PER were higher for tannin groups than CON, but with small differences between 10T and 20T. In the third ration, a similar trend was noticed as in the first one. Conversions (FC, CP, energy) were best, and KR and PER highest in 20T group. By observing the overall experiment, it is evident that the inclusion of chestnut tannins in concentrations of 20 g/kg DM expressed prime effects on FC, CP and energy conversion (4.4 kg/kg gain, 628 g/kg gain, 27.6 MJ/kg gain, respectively), as well on KR and PER (15.6 and 1.6, respectively).

Table 6 shows growth performances of lambs according to rations and the whole experiment. In the first ration, ADG for 20T were significantly higher ($p < 0.05$) than CON, but without a 10% difference. In the second ration, ADG for both 10T and 20T were similar and significantly higher ($p < 0.05$) than CON. In the third ration, there were differences, however, statistical significance was lacking, thus showing great intragroup variation. Nevertheless, if the effect of tannins on ADG for the whole experiment is considered, the concentration of 20 g chestnut tannins/kg DM improved ADG significantly ($p < 0.05$) over the CON group, while 10T did not significantly differ among the two other groups.

Dry matter digestibility was highest for CON, although CON and 10T had similar digestibility in the third ration. The digestibility of organic matter showed a similar trend, with a difference in the first ration, where values for CON and 10T were similar. In the first ration, CP digestibility was similar for CON and 10T, and higher than 20T, while in the second and third ration, CP digestibility was highest in CON. In all applied rations, higher apparent digestibility for EE were observed in 20T than in CON, as shown in Table 7.

4 Discussion

To examine whether the added tannins improved nutrient utilization, the experiment was set up to ensure that, within the experimental period, all groups had the same intake of DM, CP, and energy. This was achieved by providing the three groups with the same amount of DM which had different tannin concentrations, as shown in Table 4. As stated

Tab. 4. Average intake of dry matter, crude protein and energy
Durchschnittliche Aufnahmen an Trockenmasse, Rohprotein und Energie

Rations	Group	DMI (kg/day)	DMI (% BW)	CP consumed (g/day)	Energy consumed (MJ net energy/day)
Ration I	CON	0.8	3.0	117	5.0
	10T	0.8	3.1	117	5.1
	20T	0.8	3.0	120	5.0
Ration II	CON	1.0	3.4	140	6.3
	10T	1.0	3.3	142	6.3
	20T	1.0	3.2	141	6.2
Ration III	CON	1.1	3.4	165	7.6
	10T	1.2	3.4	169	7.5
	20T	1.2	3.3	164	7.5
Whole experiment	CON	1.0	2.9	141	6.3
	10T	1.0	2.9	143	6.3
	20T	1.0	2.8	141	6.2

DMI – dry matter intake, expressed as kg of DM per day (DMI kg/day) or % of body weight (DMI % BW); CP consumed – intake of crude protein expressed as g/day; Energy consumed – consumption of energy, expressed as MJ/day.

before, to maintain the same dry matter intake (DMI) among groups, the quantity of offered feed was based on the group that had the lowest intake on the previous day, with a daily increase to maintain feed refusals of 10%. Tannins can have astringent, an adverse taste which can lead to lower palatability and intake (TAGHIZADEH and BASHARATI, 2011), but this effect was not observed during this study. If the tannins lowered palatability, the tannin-fed groups would be characterized by the lower intake. However, on the contrary, during the whole period of the experiment (60 days), the group that had the lowest intake was CON. This excludes the adverse taste of chestnut tannins in rations. This is in accordance with the study conducted by LIU et al. (2011) who found that the addition of chestnut tannins in concentrations of 1 and 3% DM did not affect DMI.

Nutrient digestibility of the groups showed lower digestibility for CP in tannin groups than in CON, as shown in Table 7. These results are in line with the study of WISCHER et al. (2014) where 0, 43.5 and 83.4 g chestnut product/kg ration were used, and which concluded that chestnut tannins did not affect the digestibility of CF, NDF and ADF, but can significantly decrease the digestibility of CP. The main benefit of tannin inclusion in ruminant rations is the formation of tannin-protein complex, which reduces the degradation of dietary protein in the rumen (NIDERKORN et al., 2011), and, consequently, can increase intestinal flow of the amino acids released during dissociation of the mentioned complex in abomasum and duodenum (MUELLER-HARVEY, 2006; NAUMANN et al., 2017). Lower CP digestibility in tannin groups is the result of a higher amount of CP in fecal matter, and can be explained by lower digestibility (BURGGRAAF and SNOW, 2011), or by the increased flow of nitrogen in the intestines (PEREZ MALDONADO and NORTON, 1996). This

Tab. 5. Average conversion, Kleiber ratio and protein efficiency ratio
Durchschnittlicher Umsatz, Kleiber-Verhältnis und Protein-Effizienz-Verhältnis

Rations	Group	FC (kg/kg gain)	CP conversion (g/kg gain)	Energy conversion (MJ net energy/kg gain)	Kleiber ratio (KR)	Protein efficiency ratio (PER)
Ration I	CON	4.3	641	27.5	16.6	1.6
	10T	4.2	600	26.0	18.4	1.9
	20T	3.6	536	22.2	19.8	2.0
Ration II	CON	6.1	878	39.4	14.5	1.3
	10T	4.5	637	28.2	17.1	1.7
	20T	4.6	637	28.1	17.4	1.6
Ration III	CON	8.1	1180	43.1	14.5	1.1
	10T	7.4	1076	47.9	12.2	1.0
	20T	5.5	760	34.6	15.3	1.4
Whole experiment	CON	5.3	768	34.3	13.6	1.4
	10T	4.9	703	31.0	14.6	1.5
	20T	4.4	628	27.6	15.6	1.6

FC – feed conversion ratio, expressed as kg feed per kg gain; CP conversion – conversion of crude protein, expressed as g of consumed crude protein per kg gain

means that lower digestibility for CP does not necessarily indicate lower protein utilization.

On the other hand, in all three rations, EE digestibility was higher for 20T than for CON. These results are similar to the effect established in the study by WISCHER et al. (2014), where these effects were reported only for the highest chestnut concentration. Tannins are known to be bioactive in rumen, and can modify rumen fermentation and biohydrogenation, which can lead to changes in fatty acid metabolism (SARNATARO and SPANGHERO, 2020).

Different studies examining the effects of chestnut tannins in ruminant production show contradictory results. In the study that compared rations containing 4% additional tannins from different sources (originating from mimosa (*Acacia mearnsii*, De Wild), chestnut (*Castanea sativa*, Mill) or tara (*Cesalpinia spinosa*, Kuntze)) with a control ration (without added tannins) for lambs, it was observed that the lowest gains and most unfavorable conversions were in those animals that received chestnut tannin (VALENTI et al., 2019). Adding chestnut tannins to lamb rations in concentrations of 0.5, 1 and 3% of DM, did not affect DMI, ADG and FC (LIU et al., 2011; 2016). By comparing the rations with chestnut tannins in the amount of 20.8 g of product/kg of ration, and the control that did not contain tannins, no differences were detected in growth, feed conversion and length of fattening period in lambs (FRUTOS et al., 2004). Tannins from chestnut (0.6% DM), quebracho (0.6% DM) or a mixture of chestnut and quebracho (ratio 1:1, 0.6% DM) in beef steers rations in the final stage of fattening contributed to higher gains with higher energy intake (RIVERA-MENDEZ et al., 2017). The study that examined mimosa

Tab. 6. Growth performances of lambs in rations and whole experiment
Wachstumsleistungen von Lämmern in Diäten und im gesamten Experiment

Parameter/Group	CON	10T	20T	Signif.
Initial BW, kg	22.3 ± 3.0	22.2 ± 3.3	22.1 ± 2.0	NS
Final BW, kg	33.7 ± 3.7	34.8 ± 5.5	35.8 ± 2.9	NS
ADG I Ration, g	189 ^a ± 33.3	218 ^{a,b} ± 72.7	233 ^b ± 45.7	< 0.05
ADG II Ration, g	178 ^a ± 57.9	240 ^b ± 64.8	230 ^b ± 51.1	< 0.05
ADG III Ration, g	203 ± 65.5	175 ± 50.0	223 ± 43.2	NS
ADG I-III, g	190 ^a ± 35.8	211 ^{a,b} ± 47.2	228 ^b ± 27.8	< 0.05
Total gain, kg	11.4 ^a ± 2.2	12.7 ^{a,b} ± 2.8	13.7 ^b ± 1.7	< 0.05

Initial BW – lamb body weight at the beginning of the experiment; Final BW – lamb body weight at the end of the experiment; ADG – average daily gain of lambs, in first (I), second (II), third (III) ration or in whole experiment (I-III)

Means with no letter in common are significantly different (LSD-test; $\alpha = 5\%$)

and chestnut tannins in fattening cattle performance, showed no differences in ADG. The groups received rations with 14.9 g of tannin product/kg DM (mimosa (*A. mearnsii*) or chestnut (*C. sativa*)), while the control ration did not contain tannins (KRUEGER et al., 2010). Similar results were obtained by MEZZOMO et al. (2016), who concluded that the addition of a mixture of different tannin sources (quebracho (*Schinopsis* sp.), chestnut (*C. sativa*) and tara (*C. spinosa*)) in different concentrations of fattening cattle rations did not lead to a difference in ADG compared to the control group, but affected the reduction of dry matter intake, and improved conversion. In terms of PER, the inclusion of 20 g/kg DM chestnut tannins can improve overall protein and amino acid utilization.

However, none of these studies examined the influence of chestnut tannins in conditions of MP deficiency. Based on the CVB (2018) recommendations, the design of the experiment expected an ADG of 200–300 grams. In this study, rations were formulated to be 15% deficient in MP. Metabolizable protein deficiency caused the CON group to achieve lower ADG than expected (190 g/day), while tannin groups had an ADG over 200 grams (211 g and 228 g for 10T and 20T, respectively). This indicates that chestnut tannins are capable of binding with dietary proteins in complexes that are less degradable within the rumen. Because of that, growth performance responses to supplemental tannins have been generally attributed to enhancements in intestinal metabolizable protein supply (RIVERA-MENDEZ et al., 2017). In other words, chestnut tannins can have an effect on increasing the MP, enabling the animals to achieve the desired gains in MP deficiency.

The results of this study showed, that not only do tannins increase the flow of proteins to the post-ruminal parts of the digestive system, but they can also affect its better utilization, which can lead to higher performances in lambs.

5 Conclusion

The results of this study showed that chestnut tannins can have a positive effect in the utilization of rations and performances of finishing lambs in MP deficiency. The optimal

Tab. 7. Average apparent digestibility of nutrients
Durchschnittliche scheinbare Verdaulichkeit von Nährstoffen

Apparent digestibility	GROUP		
	CON	10T	20T
Collection 1			
Dry matter, %	84.0 ± 2.9	83.9 ± 2.5	80.7 ± 6.2
Organic matter, %	85.2 ± 2.7	85.4 ± 2.1	82.1 ± 5.9
Crude protein, %	78.4 ± 4.2	78.4 ± 4.5	69.0 ± 11.1
Ether extract, %	83.5 ± 2.6	89.2 ± 1.0	90.4 ± 3.1
Crude fiber, %	69.5 ± 5.7	71.3 ± 4.2	69.7 ± 10.7
Neutral detergent fiber, %	79.4 ± 3.9	80.1 ± 2.7	76.3 ± 7.8
Acid detergent fiber, %	72.2 ± 10.0	75.4 ± 3.9	69.7 ± 9.9
Collection 2			
Dry matter, %	74.0 ± 2.1	68.8 ± 6.7	71.8 ± 7.6
Organic matter, %	75.8 ± 1.8	72.4 ± 6.1	74.9 ± 6.7
Crude protein, %	70.8 ± 1.5	65.7 ± 7.8	63.2 ± 10.6
Ether extract, %	82.5 ± 2.9	81.5 ± 5.0	89.7 ± 4.8
Crude fiber, %	47.7 ± 5.0	49.2 ± 11.3	54.9 ± 11.9
Neutral detergent fiber, %	67.1 ± 2.9	65.8 ± 7.9	69.0 ± 9.0
Acid detergent fiber, %	61.0 ± 3.6	54.7 ± 10.2	60.1 ± 11.7
Collection 3			
Dry matter, %	75.7 ± 4.3	75.1 ± 3.9	72.9 ± 6.6
Organic matter, %	79.1 ± 4.0	78.4 ± 3.2	76.2 ± 5.8
Crude protein, %	68.4 ± 6.6	66.2 ± 6.7	59.4 ± 9.2
Ether extract, %	71.1 ± 6.6	77.9 ± 3.6	79.9 ± 3.4
Crude fiber, %	71.7 ± 5.3	69.7 ± 4.3	68.0 ± 8.7
Neutral detergent fiber, %	79.1 ± 4.0	79.0 ± 3.1	76.9 ± 6.3
Acid detergent fiber, %	74.7 ± 4.0	73.5 ± 4.0	68.8 ± 7.2

concentration of chestnut tannins in this study was a 20 g/kg DM ration of Farmatan Plus®. Chestnut tannins could be included in a strategy for the better utilization of proteins in ruminant nutrition, however further research is necessary.

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6 References

- ALI, M., H.A. MEHBOOB, M.A. MIRZA, H. RAZA and H. OSREDKAR (2017): Effect of hydrolysable tannin supplementation on production performance of dairy crossbred cows. *J. Anim. Plant Sci.* **27**, 1088–1093.
- AOAC (2000): Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, USA.
- BURGGRAAF, V.T. and V.O. SNOW (2011): Tannins, Types, Food Containing, and Nutrition. Nova Sci. Inc., USA.
- CHAICHISEMSARI, M., N. MAHERISIS, M. SADAGHIAN, B. ESHRATKHAH and S. HASSANPOUR (2011): Effects of administration of industrial tannins on nutrient excretion parameters during naturally acquired mixed nematode infections in Moghani sheep. *J. Am. Sci.* **7245–7252**.
- COMANDINI, P., M.J. LERMA-GARCIA, E.F. SIMO-ALFONSO and T.G. TOSCHI (2014): Tannin analysis of chestnut bark samples (*Castanea sativa* Mill.) by HPLC-DAD-MS. *Food Chemistry* **157**, 290–295.
- CVB (2018): CVB Table Booklet of Ruminants 2016. Federatie Nederlandse Diervoderketen, Nederlands.
- DE S.COSTA, E.I., C.V.D.M. RIBIERO, T.M. SILVA, R.D.X. RIBIERO, J.F. VIERIA, A.G.V. DE O.LIMA, A.M. BARBOSA, J.M. DA SILVA JUNIOR, L.R. BEZERRA and R.L. OLIVEIRA (2020): Intake, nutrient digestibility, nitrogen balance, serum metabolites and growth performance of lambs supplemented with *Acacia mearnsii* condensed tannin extract. *Anim. Feed Sci. Tech.* **272**, 114744.
- DEAVILLE, E.R., D.I. GIVENS and I. MUELLER-HARVEY (2010): Chestnut and mimosa tannin silages: effect in sheep differ for apparent digestibility, nitrogen utilisation and losses. *Anim. Feed Sci. Tech.* **157**, 129–138.
- EFSA (2005): Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on a request from the Commission on the safety and efficacy of the product Farmatan for rabbits and piglets. *The EFSA Journal* **222**, 1–20.
- FRUTOS, P., M. RASO, G. HERVAS, A.R. MONTECON, V. PEREZ and F.J. GIRALDEZ (2004): Is there any detrimental effect when a chestnut hydrolysable tannin extract is included in the diet of finishing lambs? *Anim. Res.* **53**, 127–136.
- HASSANPOUR, S., N. MAHERI-SIS, B. ESHRATKHAH and F.B. MEHMANDAR (2011): Plants and secondary metabolites (tannins): a review. *Int. J. Forest, Soil, Erosion* **1**, 47–53.
- ISO (2005): Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein content – Part 2: Block digestion/steam distillation method. ISO 5983-2, 14 pp.
- JERONIMO, E., C. PINHEIRO, E. LAMY, M.T. DENTINHO, E. SALES-BAPTISTA, O. LOPEZ and F. CAPELLA ESILVA (2016): Tannins: Biochemistry, Food Sources and Nutritional Properties, Nova Sci., Inc., USA.
- KRUEGER, W.K., H. GUTIERREZ-BANUELOS, G.E. CARSTENS, B.R. MIN, W.E. PINCHAK, R.R. GOMEZ, R.C. ANDERSON, N.A. KRUEGER and T.D.A. FORBES (2010): Effects of dietary tannin source on performance, feed efficiency, ruminal fermentation and carcass and non-carcass traits in steers fed a high grain diet. *Anim. Feed Sci. Tech.* **159**, 1–9.
- LIU, H., V. VADDELLA and D. ZHOU (2011): Effect of chestnut tannins and coconut oil on growth performance, methane emission, ruminal fermentation, and microbial populations in sheep. *J. Dairy Sci.* **94**, 6069–6077.
- LIU, H., K.LI, L. MINGBIN, J. ZHAO and B. XIONG (2016): Effect of chestnut tannins on the meat quality, welfare, and antioxidant status of heat stressed lambs. *Meat Science* **116**, 236–242.
- MAHACHI, L.N., O.C. CHIKWANHA, C.L. KATIYATIYA, M.C. MARUFU, A.O. AREMU and C. MAPIYE (2020): Sericea lespedeza (*Lespedeza juncea* var. *sericea*) for sustainable small

- ruminant production: feed, helminth suppressant and meat preservation capabilities. *Anim. Feed Sci. Tech.* **270**, 114688.
- MAKKAR, H.P.S. (2000): Quantification of Tannins in Tree Foliage - A Laboratory Manual. A joint FAO-IAEA working document, Vienna, Austria.
- MEZZOMO, R., P.V.R. PAULINO, M.M. BARBOSA, T. DA SILVA MARTINS, M.F. PAULINO, K.S. ALVES, D.I. GOMES and J.P.O. DOS SANTOS MONNERAT (2016): Performance and carcass characteristics of young cattle fed with soybean meal treated with tannins. *J. Anim. Sci.* **87**, 775–782.
- MUELLER-HARVEY, I (2006): Unravelling the conundrum of tannins in animal nutrition and health. *J. Sci. Food Agri.* **86**, 2010–2037.
- NAKAMURA, Y., S. TSUJI and Y. TONOGAI (2003): Method for analysis of tannic acid and its metabolites in biological samples: application to tannic acid metabolism in the rat. *J. Agri. Food Chem.* **51**, 331–339.
- NAUMANN, H.D., L.O. TEDESCHI, W.E. ZELLER and N.F. HUNTLEY (2017): The role of condensed tannins in ruminant animal production: advances, limitations and future directions. *Revista Brasileira de Zootecnia* **46**: 929–949.
- NIDERKORN, V., R. BAUMONT, A. LE MORVAN and D. MACHEBOUF (2011): Occurrence of associative effects between grasses and legumes in binary mixtures on *in vitro* rumen fermentation characteristics. *J. Anim. Sci.* **89**, 1138–1145.
- PATRA, A.K. and J. SAXENA (2011): Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J. Sci. Food Agri.* **91**, 24–37.
- PEREZ MALDONADO, A. and B.W. NORTON (1996): The effects of condensed tannins from *Desmodium intortum* and *Calliandra calothyrsus* on protein and carbohydrate digestion in sheep and goats. *British J. Nutr.* **76**, 515–533.
- RIVERA-MENDEZ, C., A. PLASCENCIA, N. TORRENTERA and R.A. ZINN (2017): Effect of level and source of supplemental tannin on growth performance of steers during the late finishing phase. *J. Appl. Anim. Res.* **45**, 199–203.
- SARNATARO, C. and M. SPANGHERO (2020): *In vitro* rumen fermentation of feed substrates added with chestnut tannins or an extract from *Stevia rebaudiana* Bertoni. *Anim. Nutr.* **6**, 54–60.
- STATSOFT (2003): Statistica for Windows, release 7.0, Inc., Tulsa, OK, USA.
- TAGHIZADEH, A. and M. BASHARATI (2011): Tannins, Types, Foods Containing, and Nutrition. Nova Sci., Inc.
- VALENTI, B., A. NATALELLO, V. VASTA, L. CAMPIDONICO, V. ROSCINI, S. MATTIOLI, M. PAUSELLI, A. PRIOLO, M. LANZA and G. LUCIANO (2019): Effect of different dietary extracts of lamb growth performances and meat oxidative stability: comparison between mimosa, chestnut and tara. *Animal* **13**, 435–443.
- VALENTI, B., L. CAMPIDONICO, A. NATALELLO, M. LANZA, S. SALAMI, A. PRIOLO, A. SERRA, M. PAUSELLI and G. LUCIANO (2020): Fatty acids in rumen and meat of lambs fed with different condensed and hydrolysable tannin extracts. *Research Square*, DOI: 10.21203/rs.3.rs-119707/v1.
- VAN SOEST, P.J., J.B. ROBERTSON and B.A. LEWIS (1991): Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **74**, 3583–3597.
- WISCHER, G., A.M. GREILING, J. BOGUHN, H. STEINGASS, M. SCHOLLENBERGER, K. HARTUNG and M. RODEHUTSCORD (2014): Effect of long-term supplementation of chestnut and valonea extract on methane release, digestibility and nitrogen extraction in sheep. *Animal* **8**, 938–948.