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THE INFLUENCE OF DIETARY HYDROLYZABLE TANNIN LEVELS ON RUMEN METABOLISM USING RUSITEC

(With 5 Tables and 2 Figures)

By

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تأثير التانين على ميتابوليزم الكرش باستخدام الكرش الصناعي

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الهدف من هذه الدراسة هو تقدير تأثير مستويات التانين (٢,٧ ، ٤ ، ٥,٤ ، ٨%) على درجة الأس الهيدروجيني، إنتاج الغاز ، الأمونيا – نيتروجين ، الأحماض الدهنية الطيارة ومعاملات الهضم بإستخدام سائل الكرش وتكنيك الكرش الصناعي. كان الغذاء المستخدم ١١جم دريس ، ٩جم مواد مركزة. أدى إضافة التانين إلى زيادة في درجة الأس الهيدروجيني خاصة عند مستوى ٥,٤% تانين ($P<0.01$). في حين انه قيمة معامل الإختزال لم تتغير. إنخفض تركيز الأمونيا– نيتروجين بإضافة التانين. إضافة التانين أدت الى قلة في تركيز كل من حامض الأسيتيك ، البروبيونك، والأيزوبيوتيرك ، في حين إزداد تركيز ن– بيوتيرك في معظم مستويات التانين . ولكن تركيز الأيزوفاليرك لم يتأثر بإضافة التانين. إزداد ($P<0.01$) تركيز الهكسان نتيجة لإضافة ٨% تانين . التانين له تأثير سلبي على معاملات الهضم معمليا خاصة البروتين الخام. أدت إضافة التانين بمستويات ٢,٧ ، ٤ ، ٥,٤ ، ٨% إلى إنخفاض هضم البروتين بواسطة ١١,٢ ، ١٢,٤ ، ٨,٥ ، ٢١,٥ وحدة على التوالي. الخلاصة: عند إضافة التانين في العليقة خاصة المستوى المرتفع فإنه تنخفض نواتج الهضم النهائية

ومعاملات الهضم بإستخدام الكرش الصناعي ولكن لتأكيد هذه النتائج يجب عمـــل دراســـات على الحيوانات الحية.

SUMMARY

The objective of this study was to determine the effects of dietary tannin levels (2.7, 4.0, 5.4 and 8.0%) on pH, gas production, ammonia-N, VFA's and in vitro-digestibilities in rumen using cattle's rumen liquor in rumen simulation technique (RUSITEC). The feed used was the same in two vessels, 11g hay and 9g concentrates. Addition of dietary tannin increased pH values, particularly with 5.4% tannin ($P<0.01$). While Redox values did not significantly differ among treatments. Dietary tannin reduced ammonia-N concentrations. Tannin addition decreased concentrations of acetic, propionic and I-buteric, while increased the concentration of N-buteric in most tannin's levels. However, I-valeric concentration was unaffected. Hexan concentration increased ($P<0.01$) due to 8 % tannin addition. Tannins appear to exert a negative effect on invitro-digestibilities, particularly crude protein. The decrease in crude protein digestibility due to 2.7, 4.0, 5.4 and 8.0 % tannin addition were 11.2, 12.2, 8.5 and 21.0 units, respectively. In conclusion, Dietary tannin, particularly high level, the end product of digestion and in vitro-digestibilities are decreased. To confirm these results, in vivo additional studies are necessary.

Keywords: *Rumen metabolism, In vitro-digestibilities-RUSITEC, tannin.*

INTRODUCTION

Tannins are water-soluble bio-active natural organic compounds, with a molecular weight between 500 and 3000 daltons. There are two groups of tannins, hydrolyzable tannin and condensed tannin, both are phenolic compounds and plant origin. The total soluble polyphenols in leaves of tannin containing plants which are widely spread in Upper Egypt, ranged from 10.27 to 35.46 % and the condensed tannins from 0.5 to 8.28 % on dry matter basis (Setohy, 1995). Condensed tannins are flavonoid polymers, with carbon-carbon bonds joining the individual flavoniod monomers (Porter et al., 1986), while hydrolyzable tannins are gallic or hexohydroxy diphenic acid esters of glucose or other polyols.

The ester bonds are acid-, base-, and enzyme-labile. Condensed tannins are not susceptible to hydrolysis but can oxidatively degraded in strong acid to yield anthocyanidins (Porter et al., 1986), while hydrolyzable tannins are easily broken down to gallic acid or hexahydroxydiphenic acid subunits and the core polyol (Hagerman and Butler, 1989). Tannins have harmful nutritional effects, resulting in a higher feed / weight gain in monogastric farm animals (Butler and Bos, 1993). This result may be due to that tannin complex strongly with dietary protein, and inhibited its digestion (Laurena et al., 1984), and inhibited digestive enzymes. Tannins reduced the activity of chymotrypsin, trypsin (Jansman and Longstaff, 1993), amylase (Jansman et al., 1994), carboxymethyl cellulase, protease and glutamate dehydrogenase (Ravidra and Vaithiyanathan, 1996), maltase and sucrase (Villanuera et al., 1987). However, little information is available on the effects of hydrolyzable tannin on rumen metabolism. The development and use of in vitro techniques to measure antinutritional effects of tannin was suggested for the future research by Jansman and Longstaff (1993). Therefore the objective of this study was to examine the influence of different levels of hydrolyzable tannin on rumen metabolism, namely, pH, gas volume, ammonia-N and VFA's concentrations and in vitro-digestibilities using rumen simulation technique (RUSITEC).

MATERIAL and METHODS

This study was carried out in institute of Nutrition, Veterinary Medicine University, Vienna, Austria. The objective of this study was to determine the effects of dietary hydrolyzable tannin on rumen metabolism in rumen simulation technique (RUSITEC) using cattle's rumen liquor. Tannin was pure hydrolyzable tannin from Italy.

Incubation Procedure:

Rumen simulation technique (RUSITEC, made by Wills, Ayr Scotland) was used in the present study. Outline of a typical unit of the RUSITEC taken from Czerkawski and Breckenridge (1977) is shown in Fig 1. Before feeding on the first day of incubation, 500 ml rumen fluid and 80g solid rumen content, weighed into a nylon bag with mesh 200 µm (LAT- labor und Analysen- Technik GmbH, Garbsen, Germany), were taken from fistulated cow and were placed in two reaction vessels. A bag of feed (9-g concentrate and 11-g hay) was placed inside the feed container, with a bag of solid rumen content. Two hundred ml artificial

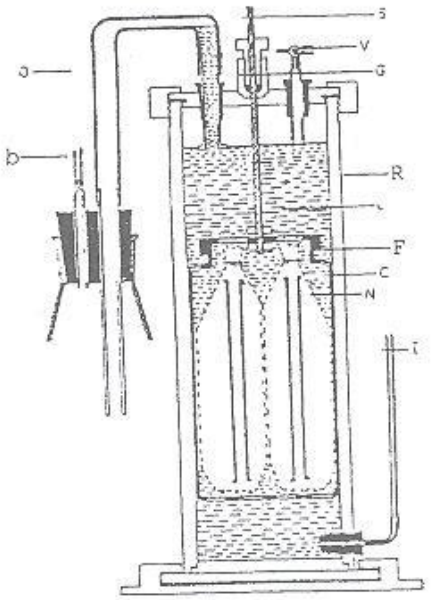

Fig 1. Outline of a typical unit in the rumen simulation technique (RUSITEC) taken from Czerkawski and Breckenridge (1977). B, to 5 l gas bag; F, feed container; L, liquid reaction mixture; N, nylongauza bag with solid digesta; G, gland; R, reaction vessel, S, moving shaft; V, sampling vent; C, moving perforated container; I, saliva input; O, efflunt output.

saliva (McDougall, 1948) were added in each vessel and the vessel was closed. The bayonet, fitting was engaged and the effluent collection flask containing 40 ml diluted Hcl (1:1), for killing microorganisms (Mauruschat, 1996), was connected to the overflow tube. RUSITEC was flushed using N_2. The three - way tap was closed and an empty gas bag (Linde plastigas, No: 37660006, Germany) was connected to the gas outlet tube. The artificial saliva (pH, 8.4) was infused (750 ml buffer in 24 h) by means of a two-channel peristaltic pump (Manostat, New York, USA) through an opening at the vessel's bottom, one for each vessel.

In the following morning at 7.0 h, the infusion was stopped and the gas bags were removed for measurement of volume using gas meter. The agitating arrangement was stopped and the reaction vessels were removed from the water- bath and new bags of feed were introduced. The feed bags were identified by using a system of attaching different colored plastic binders. The removed feed bags were washed by using 40 ml artificial saliva and the combined washings were poured back into the reaction vessels. Then the system was flushed and closed as before and the infusion of artificial saliva started. The residual material in each feed bag was dried, ground and stored for analysis. This procedure was repeated every morning, at same time, during the experiment.

Experimental and analytical procedures:

The control feed used was the same in the two vessels, 11g hay and 9 g concentrates. Chemical analyses of hay and concentrates are presented in Table 1. Four tannin's levels (2.7, 4.0, 5.4 and 8% of DM) were used. Each treatment consisted of 5 days, control followed by other 5 days treatment. Experimental plan and parameters under investigation are summarized in Table 2. The incubation procedure lasted for 2 periods, each of 32 days.

Table 1. Analyses of hay and concentrates.*

Item	Hay	Concentrates
Crude protein	12.69	20.93
Crude fat	2.10	1.48
Crude fiber	35.33	4.73
Nitrogen free extract	42.74	67.56
Ash	7.14	5.30
Organic matter	92.86	94.7

* Calculated as DM basis.

Table 2. Experimental plan and parameters under investigation. *

Day	Vessel 1	Vessel 2	Parameters
1-7	Adaptation period	Adaptation period	pH, Redox
8-12	Contol diet (CD)	Control diet (CD)	pH, Redox, NH_3, VFA's, TS, Weende
13-17	CD + 2.7% Tannin (T)	CD + 4 % Tannin (T)	PH, Redox, NH_3, VFAs, TS, Weende
18-22	CD	CD	pH, Redox, NH_3, VFA's, TS, Weende
23-27	CD + 5.4% T	CD + 8 % T	pH, Redox, NH_3, VFA's, TS, Weende
28-32	CD	CD	pH, Redox, NH_3, VFW's, TS, Weende

Where, Wend = determination of crude protein, crude fat, crude fiber and ash, TS, total solids, Gas v = Gas volume, CD = control diet, T=Tannin.

* parameters under investigation were measured daily.

About 20 ml from each vessel was taken in the morning before feed incubation for measuring pH, redox pot., NH_3-N and VFA's concentrations. Redox potentials were measured using a digital pH-meter (Type c G841, Germany) using special electrode (Mettler DM 140-Sc). The ammonia-N concentration was measured using microprocessor pH / ion Meter (PMX 3000, Germany). Volatile fatty acids (VFA's) concentrations in the effluent were determined using Gas liquid chromatograph appartus (Ranfft, 1973). The chemical analysis of feed and residual material in each feed bag was carried out by standard methods (Neumann and Bassler, 1976).

The number of measurements used for individual statistical comparisons between control and treatment was 10, two periods of 5 days. Each tannin's level treatment consisted of 5 days control and other 5 days treatment. Data were analyzed using General linear Model (GLM) procedure (SAS, 1987) for personal computer.

RESULTS and DISCUSSION

pH, Redox, gas volume and NH_3-N:

Table 3 illustrates the pH, Redox, gas production and NH_3-N concentration in RUSITEC fed different levels of tannin. The pH value tended to be higher in most tannin levels. Addition of 5.4 % hydrolyzable tannin increased ($P< 0.01$) pH value (6.77 vs. 6.61). This results can be regarded as an indicator of negative effects of tannins on fermentation and VFA`S production. Makkar et al. (1988) reported that tannin could reduce the activity of ruminal microflora and activity of microbial enzymes. Dong et al. (1998) stated that the phenolic hydoxyl

groups of the active tannins appear to play an important role in their inhibitory effect on the enzymes. Redox values and gas volume did not significantly affected by tannin treatments (Table 3).

Table 3. pH, Redox, gas volume and NH_3-N concentration in RUSITEC as influenced by dietary tannin.

Item	Tannin level								
	2.7%		4%		5.4%		8%		
	C	T	C	T	C	T	C	T	SE
PH	6.77	6.78	6.76	6.80	6.61[a]	6.77[b]	6.77	6.80	0.03
Redox	342.60	346.30	338.5	338.80	339.30	337.90	345.8	344.6	4.80
Gas volume,l	2.29	2.29	2.49	2.51	2.25	2.27	2.41	2.43	0.11
NH_3-N mg/dl	17.73[c]	15.26[d]	18.94[c]	16.67[d]	19.17[c]	16.63[d]	17.73	17.65	0.83

Values are least-squares means (LSM) and SE=standard error of LSM, Treatments: C=control; T=tannin; a,b ($P<0.01$), c,d ($P<0.05$)

Dietary tannin decreased the concentration of NH_3-N. Ammonia nitrogen concentration decreased ($P< 0.05$) by about 14, 12 and 13 % due to 2.7, 4.0 and 5.4 % tannin treatments, respectively. Tannin protected dietary protein from degradation (Laurena et al., 1984), which is a main factor determining NH_3 concentration in the rumen liquor. Inhibition of microbial growth or microbial protein synthesis, by adsorption of tannin to cell membranes (Menke et al., 1992), consequently NH3-N uptake may involve.

Volatile fatty acids (VFA's) concentration:

Table (4) shows the effects of different levels of hydrolyzable tannin on VFA's concentration. Tannin addition decreased concentrations of VFA's, i. e acetic, propionic, I-buteric and N-valeric in most tannin's levels. Such decreases were significant ($P<0.05$) for propionic (5.4% tannin), I-Buteric (8% tannin) and N-valeric (5.4 and 8 % tannin). I-Valeric concentration was unaffected by dietary tannin. N-Buteric concentration tended to be higher in most tannin's levels, such increase was only significant with 8% tannin. Hexan concentration tended to be lower in all tannins levels with exception that of 8%, where it was significantly ($P<0.01$) higher with treated diet than with control diet (Table 4).

Table 4. VFA concentrations (mol, %) in RESITEC as influenced by dietary tannin.

Item	Tannin level								
	2.7%		4%		5.4%		8%		
	C	T	C	T	C	T	C	T	SE
Acetic	51.71	48.19	55.46	53.02	53.61	54.48	53.28	50.30	2.00
Propionic	22.60	21.45	21.84	21.27	22.29c	19.68d	20.33	18.08	0.92
I-Buteric	1.17	1.02	1.14	1.13	1.21	1.10	1.18c	1.00d	0.06
N-Buteric	14.74	16.03	16.18	15.94	17.09	17.45	16.68a	17.07b	0.55
I-Valeric	2.98	3.25	3.18	3.46	3.95	3.98	3.69	3.55	0.18
N-Valeric	2.82	2.75	3.10	2.78	3.08c	2.69d	2.94a	2.85b	0.14
Hexane	1.39	1.23	1.37	1.31	1.12	0.89	1.30a	1.41b	0.17

Values are least-squares means (LSM) and SE=standard error of LSM, Treatments: C=control; T=tannin ; a,b (P<0.01) c,d (P< 0.05)

This inhibition of VFA's production may be due to the precipitation of most proteins in the form of insoluble tannates and also formation of a protective layer around the starch granules. The increase of pH value due to tannin treatments (Table 3) is supportive to this view. Singh and Arora (1979) found that neither natural tannin nor added tannic acid at a level of its presence in sale seed had any significant effect on fresh VFA production. Whereas when they added at a level of 26.6 mg / ml (ten times higher level of its presence in sale seed), the VFA production was completely inhibited.

In vitro-digestibilities (in RUSITEC):

Digestibility coefficients in RUSITEC after incubation with rumen contents and fed different levels of hydrolyzable tannin are presented in Table 5. Digestibility values of all nutrients were negatively affected by dietary tannin (Fig. 2). This negative effect tended to be more pronounced with the highest level of tannin (8%). For example, the decrease in crude protein digestibility due to 2.7, 4.0, 5.4 and 8.0% tannin were 11.2, 12.2, 8.5 and 21.0 units, respectively. Similar trend was found for all other nutrients (Table 5). This result is in accordance with the previous results which pointed out lower VFA's production (Table 4) and higher pH values (Table 3) with tannin treated diet than with control diet. The adverse effects of tannin on in vitro-digestibilities may be attributed to, it forming complexes with proteins; i.e., dietary protein (Menk et al., 1993) microbial protein (Mullins and Nemith, 1988) or enzymes (Jansman et al., 1993; Jansman et al., 1994, Ravindra

and Vaithiyanathan, 1996, Konishi and Tanaka, 1999). Consequently such effect may protects protein and/or other nutrients from degradation.

Table 5. Influence of tannin's level on digestibility coefficients using RUSITEC.

Item	Tannin level								SE
	2.7 %		4.0 %		5.4 %		8.0 %		
	C	T	C	T	C	T	C	T	
Crude protein	80.72c	69.48d	80.07c	67.88d	79.07	70.56	80.90a	59.88b	3.09
Crude fat	53.12c	30.80d	50.08c	28.47d	53.41c	33.69d	51.40a	12.61b	6.45
Crude fiber	62.42	57.63	59.53	58.00	61.30	57.72	60.42	54.55	3.62
NFE	80.23	76.58	79.01	75.83	80.02	77.29	78.87	73.62	2.34
Ash	73.05c	58.55d	71.19c	56.19d	68.76	57.56	71.90a	47.20b	3.78
OM	75.68	69.89	74.14	69.41	75.02	70.75	74.43a	65.63b	2.18

Values are least-squares means (LSM) and SE=standard error of LSM. Treatments: C=control; T=tannin; a,b (P<0.01), c,d (P<0.05), e,f (P<0.10)

Fig. 2. Simple correlation between in vitro - digestibility coefficients and dietary tannin.

In conclusion, when tannin included in the diet, particularly high level, the end product of digestion and in vitro-digestibilities are negatively affected. To confirm these results, additional studies including in vivo-observations are necessary.

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