EFFECT OF DIETARY SUPPLEMENTATION OF CHESTNUT HYDROLYSABLE TANNIN ON DIGESTIVE EFFICIENCY, GROWTH PERFORMANCE AND MEAT QUALITY IN GROWING RABBITS

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ABSTRACT

The aim of the study was to evaluate the effect of the dietary supplementation of chestnut hydrolysable tannin on the feed digestibility and nutritive value, mortality and growth performance,fatty acid profile and TBARS of cooked *Longissimus dorsi* (LD) meat. From the age of 18 days the rabbits were fed pellets supplemented with coccidiostat (CC) or with tannin (400g/100 kg: T400). At weaning (35 d of age) within both groups 5 dietary sub-groups were formed: medicated-free (C0), containing coccidiostatic (CC), or supplemented with increasing levels of chestnut tannin (T200, T400 and T600). Rabbits fed the 5 diets *ad libitum* until slaughter at 11 weeks of age. The coefficient of total tract apparent digestibility (CTTAD) of nutrients was not affected by the tannin inclusion, substantially, with exception of CTTAD of Ca, lower in T400 *vs* C0 and CC ($P<0.05$), although the DP-to-DE ratio was lowest in CC and T600 ($P<0.001$). Dietary tannin significantly modified the CTTAD of FA classes.At weaning, the live weight of CC rabbits was significantly higher than that of the T400 rabbits (974 *vs* 940 g, $P<0.05$). The other variables related to growth performance, health status and carcass traits were not affected by the tannin supplementation. The T400 diet fed before weaning seemed to be more effective than the 3 tannin levels administered during fattening period on FA profile change in the LD muscle.T400 diet fed before weaning increased SFA and MUFA ($P<0.01$) and decreased PUFA ($P<0.05$), decreasing the n-6/n-3 ratio ($P<0.01$), however. When tannin-supplemented diets were fed during fattening, only T600 diet showed significantlyhigher ($P<0.05$) SFA and MUFA contents compared to CC diet. In conclusion, the dietary inclusion of chestnut hydrolysable tannin doesn't provides improvements in health status, diet nutritive value, growth performance and carcass traits. Dietary hydrolysable tannin supplementation may play a role on lipid metabolism.

Key words: Rabbit, chestnut hydrolysable tannin, feed, digestibility, live performance, meat quality

INTRODUCTION

Tannins are a heterogeneous group of phenolic polymers. According to their chemical structure they can be divided into hydrolysed tannins (HT) and condensed tannins (CT). Altogether, tannins are reported to have various physiological effects like antimicrobial, antiparasitic, antioxidant and antiradical activity (Marín-Martinez *et al.*, 2009). The bactericidal activity of CT from red quebracho tree (at inclusion level of 1.1% and 3%) was confirmed in fattening rabbits reared under moderately high environmental temperature (Dalle Zotte and Cossu, 2009). Recently, dietary HT from chestnut wood up to 1% of inclusion level were shown to improve the oxidative stability of the meat and certain stress parameters (i.e. cortisol, CK, WBC) on rabbits reared under high environmental temperatures (Liu *et al.*, 2012). The FEEDAP Panel (EFSA, 2005) by evaluating the results of 6 studies, concluded that the efficacy of HT from chestnut wood is demonstrated only on reducing signs of diarrhoea and/or lowering mortality to animals suffering from enteropathy, as further proved by Maertens and Štruklec (2006). Diets rich in tannins may reduce the feed intake and the protein digestibility (Gidenne *et al.*, 1998) because tannins precipitate protein forming of a protein/tannin complex. However the effect of a dietary inclusion of chestnut HT on nutrient apparent digestibility in the rabbit was not evaluated yet. Few studies were conducted to evaluate the effect of the dietary HT extract supplementation on the carcass and meat traits in the rabbit under thermoneutral conditions. Gai *et al.*(2009) observed that 0.5% HT reduced the meat TBARS values, but only at 30 min of induced oxidation. At a 1% inclusion level, HT showed a pro-oxidant effect and the iron content of the meat increased significantly (Liu *et al*., 2009). More recently, Dalle Zotte *et al.*(2010) found that meat color and conjugated dienes were unaffected by HT supplementation. The aim of our study was to determine if supplementing a commercial diet for growing-fattening rabbits with increasing levels of HT from chestnut wood the animal health, live performances, digestive efficiency and carcass and meat quality could be affected.

MATERIALS AND METHODS

Animals, diets, carcass traits and meat sampling

Large body line rabbits of the Pannon breeding program were used.From the age of 18 days the rabbit kits of 72 does were fed pellets supplemented with coccidiostatic (0.5% Robenidin:CC), or with hydrolysable tannins (HT) extract from chestnut wood (*Castanea sativa* Mill.) at concentration of 400 g/100 kg (T400). At weaning (35 d of age) within both groups the rabbits were randomly allocated to five diets (36 rabbits per diet): control diet (without coccidiostatic and HT: C0), CC diet, and three levels of HT(T200, T400 and T600), and housed in pairs. Experimental diets were isoprotein and isoenergy (16±0.4% crude protein, 9.83±0.08 MJDE /kg). Individual live weight and feed intake were recorded every two weeks. The health condition of the rabbits was monitored daily. Rabbits were slaughtered at 79 days of age. Carcasses were chilled for 24 h at +3 °C, then dissected, and carcass yield, perirenal fat incidence and hindleg meat/bone ratio were calculated. *Longissimus dorsi* (LD) muscle was dissected and frozen until chemical analysis.

Digestibility trial

The coefficients of total tract apparent digestibility (CTTAD)of dry matter, nutrients, minerals, fatty acid (FA) and the digestible energy (DE) concentration of each experimental diet were measured in an *in vivo* digestibility assay carried out on 40 rabbits (8 males per diet) according to the European standardized method (Perez *et al*., 1995). The digestibility trial started at 62d of age with a 4-d collection period.

Chemical analyses

Diets and faeces were analysed to determine the concentrations of dry matter, crude protein, mineral content and starch, using AOAC (2000) methods. Ether extract was analysed after acid-hydrolysis treatment. Fibre fractions were determined using the sequential procedure and the filter bag system (Ankom Technology, New York). Gross energy was determined with an adiabatic bomb calorimeter.LD meat was analysed for FA profile and TBARS value (expressed as MDA, mg/kg meat) according to Botsoglou *et al*. (1994). For TBARS determination, LD meat was previously cooked (5 min at 70 °C core temperature).Diets, faeces and HL meat were analysed for quantifying the presence of metabolites of tannic acid (gallic acid, ellagic acid and pyrogallol) related to the dietary HT supplementation according to ShuiandLeong (2004).

Statistical analysis

ANOVA (GLM procedure of SAS, 2004) tested the diet effect administered before weaning (BW= CC, T400), after weaning (AW= C0, CC, T200, T400, T600) and their interactions (BWxAW) as fixed effects.Mortality was evaluated by chi^2 test using SPSS 10.0 software package.

RESULTS AND DISCUSSION

Apparent digestibily of nutrients wasn't affected by the chestnut HT inclusion, substantially, with exception of CTTAD of Ca, lower in T400 *vs* C0 and CC ($P<0.05$), although the DP-to-DE ratio was lower in CC and T600 ($P<0.001$) (Table 1). Also in piglets the HT at 0.8% inclusion reduced P and Ca digestibility (EFSA, 2005).

Table 1. Coefficients of total tract apparent digestibility and nutritive value of experimental diets

	C0	CC	T200	T400	T600	*P* value	SE
Rabbits no.	8	8	8	8	8		
Live weight (LW)	1739	1733	1733	1768	1721	0.955	18.6
Dry matter	0.582	0.583	0.577	0.572	0.578	0.878	0.004
Crude protein	0.732	0.711	0.719	0.714	0.706	0.626	0.005
Ether extract	0.797	0.756	0.79	0.785	0.762	0.080	0.006
NDF	0.289	0.286	0.303	0.277	0.248	0.093	0.007
Starch	0.978	0.974	0.971	0.972	0.975	0.123	0.001
Gross energy	0.605	0.600	0.595	0.593	0.595	0.860	0.004
Ca	0.509^{b}	0.507^{b}	0.469^{ab}	0.443^{a}	0.495^{ab}	0.017	0.008
K	0.711	0.711	0.706	0.709	0.735	0.630	0.006
P	0.293	0.308	0.264	0.225	0.290	0.094	0.011
Na	0.666	0.610	0.579	0.605	0.550	0.601	0.023
Fe	0.008	-0.011	-0.008	-0.015	0.033	0.261	0.008
Nutritive value:							
DP, g/kg	129	123	130	131	124	0.098	1.07
DE, MJ/kg	11.1	10.9	10.9	10.9	10.9	0.916	0.07
DP-to-DE ratio, g/MJ	11.7^{bc}	11.2^{a}	11.9^{c}	12.0^{c}	11.4^{ab}	0.000	0.07

Means with different letters on the same row differ significantly (Bonferroni test) (P<0.05).

Table 2. Coefficients of total tract apparent digestibility of fatty acids

Means with different letters on the same row differ significantly (Bonferroni test) (P<0.05).

	C0	CC	T200	T400	T600	*P* value	SE
n	8	8	8	8	8		
ΣSFA	-0.36^{a}	-0.36^{a}	-0.47^{b}	-0.39^{ab}	-0.42^{ab}	0.027	0.013
ΣMUFA	0.56^{b}	0.57^{b}	0.51^{a}	0.54^{ab}	0.54^{ab}	0.001	0.005
ΣPUFA	0.84^{c}	0.81^{ab}	0.86^{d}	0.81^{a}	0.82^{b}	<0.001	0.003
- C18:1n-9	0.58^{c}	0.57^{bc}	0.53^{a}	0.54^{ab}	0.55^{abc}	<0.001	0.005
- C18_2n-6	0.85^{c}	0.82^{a}	0.87^{d}	0.82^{a}	0.84^{b}	<0.001	0.003
- C18_3n-3	0.75^{a}	0.79^{bc}	0.83^{d}	0.79^{b}	0.81^{c}	<0.001	0.004
- Σn-6	0.85^{c}	0.82^{a}	0.87^{d}	0.82^{a}	0.84^{b}	<0.001	0.003
- Σn-3	0.72^{a}	0.75^{b}	0.79^{d}	0.76^{bc}	0.77^{cd}	<0.001	0.004

Dietary hydrolysed tannins significantly modified the apparent digestibility of fatty acid classes (Table 2). Anyway, as faecal FA are a result of the enzymatic activity both in the small intestine and in the caecum, through caecal microflora, HT could have affected the FA metabolism at both levels. Increasing dietary chestnut HT supplementation the gallic acid content increased linearly in T200, T400 and T600 diets, but neither faeces nor HL meat contained tannic acid metabolites at detectable level (Table 3).Ellagic acid content was high in pure tannin but surprisingly low in diets, probably related to formation of polymeric forms during feed pelleting, not detectable by the method used for determining HT. In fact, it was reported that high temperature (60 °C) affects ellagitannins stability (Lei, 2002).At weaning, the live weight of CC rabbits was significantly higher than that of the T400 rabbits (974 *vs* 940 g, P<0.05). The other variables related to growth performance, health status and carcass traits were not affected by the HT supplementation (Table 4).

Table 3. Types of hydrolysable tannins in diets, faeces and hindleg meat (mg/kg fresh basis)[1]

	Gallic acid	Ellagic acid	Pyrogallol
Tannin 75%	553	2318	nd
Diet C0	nd	34.72	39.04
Diet T200	11.22	30.48	40.20
Diet T400	30.70	23.16	41.14
Diet T600	57.18	26.28	46.72
Faeces and Meat C0, T200, T400, T600	nd	nd	nd

[1] DM: 91.86, 89.82, 89.79, 89.59 and 89.88 % for Tannin 75%, Diets C0, T200, T400 and T600, respectively. nd: not detected.

Table 4. Growth performance, mortality and carcass traits

	Before weaning BW		After weaning AW					*P* value			SE
Diet	CC	T400	C0	CC	T200	T400	T600	BW	AW	BWxAW	
Rabbits, no.	169	175	70	72	67	68	67				
LW at 5 wk, g	974	940	961	958	949	963	963	0.005	0.947	0.779	6.1
LW at 11 wk, g	3033	3004	3037	3006	3002	3020	3029	0.898	0.955	0.582	0.35
WG 5-11 wk, g/d	49.0	49.1	49.4	48.8	48.8	48.9	49.4	0.341	0.933	0.647	15.7
FI 5-11 wk, g/d	151	152	152	151	151	152	150	0.748	0.953	0.343	1.0
FCR 5-11 wk	3.09	3.10	3.09	3.11	3.11	3.12	3.04	0.781	0.626	0.187	0.02
Mortality 5-11 wk, %	3.5	4.1	5.9	0	5.9	1.4	5.9	0.778	0.196	-	-
Carcass yield, % SW	61.8	61.8	61.9	61.9	61.8	61.9	61.7	0.883	0.836	0.596	0.08
Perirenal fat, % RC	1.82	1.70	1.85	1.73	1.82	1.76	1.63	0.078	0.296	0.930	0.03
Hindleg meat/bone ratio	6.59	6.66	6.62	6.38	6.64	6.67	6.81	0.576	0.328	0.762	0.06

LW: live weight; WG: weight gain; FI: feed intake; FCR: feed conversion ratio; SW: slaughter weight; RC: reference carcass. Means with different letters on the same row differ significantly (Bonferroni test) ($P<0.05$).

Table 5. Fatty acid classes (%FAME) of diets and LD meat, and TBARS (mg MDA/kg) of cooked LD meat

	Before weaning BW		After weaning AW					*P* value			SE
	CC	T400	C0	CC	T200	T400	T600	BW	AW	BW x AW	
Diets:											
ΣSFA	15.5	15.2	17.1	15.5	15.8	15.2	15.3				
ΣMUFA	22.4	22.4	22.9	22.4	22.3	22.4	21.9				
ΣPUFA	58.1	58.6	53.9	58.1	58.1	58.6	59.4				
n-6/n-3	12.8	13.0	13.5	12.8	12.8	13.0	12.6				
LD meat:											
Rabbits, no.	40	39	16	16	16	15	16				
ΣSFA	41.1	43.8	43.0^{ab}	40.2^{a}	41.0^{ab}	43.3^{ab}	44.8^{b}	0.004	0.017	0.841	0.51
ΣMUFA	24.4	26.0	25.8^{ab}	23.6^{a}	24.5^{ab}	25.8^{ab}	26.3^{b}	0.006	0.016	0.614	0.31
ΣPUFA	33.9	31.6	31.8	34.2	34.1	31.7	31.9	0.025	0.290	0.390	0.54
n-6/n-3	12.1	10.8	11.0	12.4	12.1	10.6	11.1	0.004	0.063	0.509	0.24
MDA, mg/kg	0.026	0.023	0.028	0.029	0.021	0.019	0.024	0.612	0.820	0.677	0.003

Means with different letters on the same row differ significantly (Bonferroni test) ($P<0.05$).

The T400 diet fed before weaning seemed to be more effective than the 3 tannin levels administered during fattening period on FA profile change in the LD muscle (Table 5). T400 diet fed before weaning increased SFA and MUFA ($P<0.01$) and decreased PUFA ($P<0.05$), decreasing the n-6/n-3 ratio ($P<0.01$), however. When tannin-supplemented diets were fed after weaning, only T600 diet showed significantly higher ($P<0.05$) SFA and MUFA contents compared to CC diet. The coccidiostatic could have been also responsible of differences in fatty acids digestibility and fatty acids profile in meat.

CONCLUSIONS

In conclusion, the dietary inclusion of chestnut hydrolysed tannins didn't provide improvements in health status, diet nutritive value, growth performance, carcass traits and oxidative stability of the rabbit meat.

ACKNOWLEDGEMENTS

Research funded by Padova University research funds Ex 60% 2009 (60A08-4811/09).This research was also supported by the TECH_08_A3/2-2008-0384 project by the NDA (National Development Agency). Authors thank S.Balzan, S.Tenti, M. Cullere, M.Meurant,P.Stephano, S. Zannoni for their technical support.

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