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Effects of dietary supplementation of tea saponins (*Ilex kudingcha C.J. Tseng*) on ruminal fermentation, digestibility and plasma antioxidant parameters in goats☆

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ABSTRACT

Four goats (25 ± 2.5 kg) fitted with the ruminal, duodenal and ileal cannulae were used in a 4 × 4 Latin square design experiment to determine effects of dietary supplementation of tea saponins (TS) extracted from tea leaves (*Ilex kudingcha C.J. Tseng*) on ruminal fermentation, digestibility and plasma antioxidant parameters in goats. The goats were fed the same basal diet with levels of TS supplementation of 0 (TS0), 400 (TS400), 600 (TS600) or 800 (TS800) mg TS/kg dry matter (DM). Dietary TS levels did not affect apparent disappearance in the forestomach of DM, N, neutral detergent fiber (NDFom) or acid detergent fiber (ADFom), or apparent digestibility in the small intestine of NDFom and ADFom. Ruminal pH, ammonia N and volatile fatty acid concentrations were not affected by TS addition, as was the plasma concentrations of glutathione peroxidase and malondialdehyde. However triacylglycerol level declined linearly (P<0.01) with increasing levels of TS addition, Results indicate that dietary supplementation of TS did not affect nutrient digestibility, patterns of rumen fermentation, and plasma metabolite concentrations.

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1. Introduction

Tea saponins (TS) are one of the most important active components in tea and tea seed meal and its resultant nutritional and physiological functions have encouraged its application in research and livestock production systems. Modification of rumen fermentation was achieved by addition of *Yucca schidigera* or tea saponins to heifer diets or to the substrates during *in vitro* fermentation (Hristov et al., 1999; Liu et al., 2003; Hu et al., 2005). Saponins have also been suggested to favor production of propionate in the rumen (Lila et al., 2003; Wina et al., 2005). Ye (2001) found that dietary TS supplementation

Abbreviations: AA, amino acid; ADFom, acid detergent fiber; CP, crude protein; DM, dry matter; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; ME, metabolizable energy; NDFom, neutral detergent fiber; TG, triacylglycerols; SOD, superoxidase dismutase; TS, tea saponins; VFA, volatile fatty acid.

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Table 1
Ingredient and chemical composition (g/kg DM) of the experimental diets.

Ingredient composition		Chemical composition	
Maize stover	500.0	ME (MJ/kg)	11.76
Corn	267.7	CP	132.0
Soybean meal	148.1	Starch	223.4
Fat meal	50.0	NDFom	375.0
NaCl	8.0	ADFom	255.7
Urea	6.2	Ca	2.50
Premix	20.0	P	2.40

Metabolisable energy value was reported by Zhang and Zhang (1998). Premix: g/KgDM was 243.8 g $MgSO_4 \cdot H_2O$, 15.8 g $FeSO_4 \cdot 7H_2O$, 3.3 g $CuSO_4 \cdot 5H_2O$, 13.0 g $MnSO_4 \cdot H_2O$, 14.5 g $ZnSO_4 \cdot H_2O$, 20 mg Na_2SeO_3, 60 mg KI, 40 mg $CoCl_2 \cdot 6H_2O$, 95,000 IU vitamin A, 17,500 IU vitamin D, 18,000 IU vitamin E.

improved the performance of growing lambs, and average daily gain and feed conversion efficiency were increased with daily supplementation of 3 g/d of TS in the diets of goats (Hu et al., 2006).

Natural antioxidants extracted from grape seed and tea have attracted interest as dietary supplements of small ruminants (Sgorlon et al., 2005; Zhong et al., 2009) and dietary tea catechin supplementation can affect cellular oxidation in rats (Ikeda et al., 1992). Data from our laboratory has shown that supplementation of dietary tea catechin extracted from green tea leaves can decrease lipid oxidation and improve meat quality of goats (Zhong et al., 2009).

Our objective was to determine effects of TS on ruminal fermentation, digestibility and plasma antioxidant parameters in goats.

2. Materials and methods

2.1. Preparation of tea saponins

The TS was isolated from green tea leaves (*Ilex kudingcha C. J. Tseng*) at the National Engineering Center of Botanical Functional Ingredients Utilization, Hunan Agricultural University (Changsha, China). The extract was in powder form, light-brown in color, and contained above 700 g/kg triterpenoid saponins.

2.2. Animals and management

The use of the animals and the experimental procedures were approved by the Animal Care Committee, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, China.

Four Xiangdong black wethers goats (a local breed in the South of China), with initial body weight of 25 ± 2.5 kg, were each fitted with a ruminal plastic cannula (4 cm internal diameter) and proximal duodenal and terminal ileal fistulae (T-Type, 1 cm internal diameter; Laboratory Factory, Yinchuan, Ningxia University, Ningxia, China). The goats were kept individually in stainless steel metabolism cages in a temperature controlled (21 °C) and constantly lighted animal house with free access to fresh water.

2.3. Experimental diets and design

The experiment was a 4×4 Latin square with four goats, four dietary treatments (*i.e.*, supplemented with 0 (TS0), 400 (TS400), 600 (TS600) and 800 (TS800) mg TS/kg feed dry matter (DM) and four periods of 24 d consisting of 14 d for adaptation, 7 d for small intestinal digestibility determination, 2 d for determination of rumen fermentation characters and 1 d for blood sample collection.

Diets were prepared to meet 1.4 times maintenance metabolizable energy (ME) requirements of these Chinese Goats (Lu and Zhang, 1996). The ingredient and chemical composition of the experimental total mixed rations, Table 1, were fed twice daily and refusals were collected and weighed daily for 7 d before commencement of the formal experiment to measure the voluntary feed intake. During the formal experiment, the amounts of experimental rations were fed in equal portions at 07:00 and 19:00 h daily according to previously determined voluntary feed intake to minimize feed refusals.

2.4. Sample collection and handling

The diets for each period were equally sampled, oven dried at 65 °C, air equilibrated, ground to pass a 1 mm sieve, and stored pending laboratory analysis.

From day 15 to 21 of each period, a total of 4 g chromic oxide (g every 6 h/d), as the indigestibility marker was administered daily *via* the rumen fistulae at 06:00, 12:00, 18:00 and 24:00 h (Tan et al., 2001). Thereafter, 30 ml duodenal and ileal fluid was collected at 05:00, 11:00, 17:00 and 23:00 h on day 19, at 03:00, 09:00, 15:00 and 20:00 h on day 20 and at 01:00, 07:00, 13:00 and 19:00 h on day 21, respectively. At the end of each period, equal portions of the duodenal and ileal samples were composited within goat and used to measure the digesta flows.

Ruminal fluid samples (50 ml) were collected with a rumen filter tube *via* the ruminal cannula at 0, 1, 2, 3, 5, 7, 9, 11, 12, 13, 14, 17, 21 and 24 h after morning feeding on day 22 and 23 of each period. The pH values of the fluid samples were determined and recorded using a pH meter (REX pHS-3C, Shanghai instrument factory, Shanghai, China). Samples were immediately squeezed through 4 layers of cheesecloth with a mesh size of 250 μm and 10 ml of filtered rumen fluid was centrifuged using a centrifuge (Himac CR22G2, Hitachi Koki Co., Ltd., Tokyo, Japan) at 20,000 × *g* for 15 min at 4 °C to obtain a clear supernatant for ammonia N determination. Another 10 ml was centrifuged at 500 × *g* for 10 min at 4 °C and the clear supernatant was put into a plastic bottle containing 1 ml of 0.25 g/ml metaphosphoric acid and 1 ml of 0.006 g/ml 2-ethylbutyric acid (internal standard), and further centrifuged at 20,000 × *g* for 15 min at 4 °C to obtain a final supernatant which was stored at −20 °C for volatile fatty acid (VFA) analysis.

On day 24 of each period, 10 ml blood samples were collected from the jugular vein of each goat at 1, 5, 9, 13, 17 and 21 h after the morning feeding and placed in aseptic vacutainer tubes containing Li-heparin (Becton Dickinon, Vacutainer Systems, Rutherford, NJ, USA). Blood samples were then centrifuged (Himac CR22G2, Hitachi Koki Co., Ltd., Tokyo, Japan) at 3000 × *g* for 15 min at 4 °C to harvest plasma which were stored at −20 °C until analysis of antioxidant parameters. Total feces were collected according to the procedure of Sun et al. (2007) from days 19 to 21 of each period and sub-sampled by goat for analyses.

2.5. Chemical analysis

Dry matter (oven dried at 60 °C for 48 h) and total N (#984.13) of diets, duodenal and ileal chyme were determined according to the methods of AOAC (1990). Ash-free neutral detergent fiber (NDFom) of diets, duodenal and fecal samples was determined without use of sodium sulfite or amylase according to Van Soest et al. (1991). Acid detergent fiber (ADFom) of diets, duodenal and fecal samples were determined (#973.18; AOAC, 1990) and expressed exclusive of residual ash. The amino acid (AA) content of duodenal and ileal samples were determined according to Mason et al. (1980). Chromium content of duodenal and ileal chyme and feces was determined colorimetrically after oxidation to chromate according to Schurch et al. (1950).

The ammonia concentration of ruminal fluid supernatant was analyzed by a phenol-hypochlorite assay (Chaney and Marbach, 1962). The VFA content was separated on a packed column (model SP-1200, Supelco, Bellefonte, PA, USA) with 2-ethyl butyric acid as the internal standard, and quantified by gas chromatography (Hewlett Packard 5890, Palo Alto, CA, USA).

Concentrations of triacylglycerols (TG), superoxidase dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) in plasma were analyzed by kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.6. Calculations

Flows of DM, N, NDFom, ADFom and individual AA was calculated as described by Sun et al. (2007), and the apparent digestibility of DM, N, NDFom, ADFom and individual AA was calculated as:

$$D_{\mathrm{fs}} = \frac{(F_{\mathrm{in}} - F_{\mathrm{du}})}{F_{\mathrm{in}}}$$

$$D_{\mathrm{si}} = \frac{(F_{\mathrm{du}} - F_{\mathrm{il}})}{F_{\mathrm{du}}}$$

$$D_{\mathrm{ap}} = \frac{(F_{\mathrm{in}} - O_{\mathrm{fe}})}{F_{\mathrm{in}}}$$

where D_{fs} = apparent digestibility in the forestomach; F_{in} = feed intake; F_{du} = the duodenal flow; D_{si} = apparent digestibility in the small intestine; F_{il} = the ileal flow; D_{ap} = apparent whole tract digestibility; O_{fe} = fecal output.

2.7. Statistical analysis

Data on digestibility of DM, N, NDFom, ADFom, and individual AA were analyzed using PROC GLM of SAS (1996) with the model:

$$Y_{ijk} = \mu + \mathrm{Period}_i + \mathrm{Goat}_i + \mathrm{Treatment}_k + \varepsilon_{ijk}$$

where Y_{ijk} is the response, μ is the overall mean, Period_i is the effect of period, Goat_j is the effect of goat, $\mathrm{Treatment}_k$ is the effect of dietary TS supplementation levels. Error (ε_{ijk}) was assumed independent and N (0, σ^2).

For rumen fermentation and plasma antioxidant parameters, which had repeated measures over time, the model was:

$$Y_{ijk} = \mu + \mathrm{Period}_i + \mathrm{Goat}_j + \mathrm{Treatment}_k + \mathrm{Hour}_m + \varepsilon_{ijkm}$$

Table 2
Effects of tea saponin levels on nutrients digestibility in goats.

	Treatment				P		
	TS0	TS400	TS600	TS800	SEM	L	Q
DM							
Intake, g/d	508.8	508.8	510.7	509.8	12.64	0.10	0.97
Apparent digestibility in the forestomach	0.40	0.26	0.30	0.32	0.043	0.23	0.11
Apparent digestibility in the small intestine	0.28	0.25	0.16	0.22	0.037	0.13	0.57
Apparent whole tract digestibility	0.64	0.56	0.60	0.59	0.024	0.57	0.32
N							
Intake, g/d	11.61	12.49	10.97	12.19	0.923	0.91	0.97
Apparent digestibility in the forestomach	0.23	0.26	0.23	0.26	0.017	0.42	0.65
Apparent digestibility in the small intestine	0.51	0.49	0.45	0.45	0.063	0.36	0.78
Apparent whole tract digestibility	0.80	0.83	0.84	0.84	0.043	0.43	0.58
NDFom							
Intake, g/d	197.1	196.1	197.2	197.1	6.30	0.78	0.38
Apparent digestibility in the forestomach	0.47	0.44	0.44	0.43	0.015	0.28	0.61
Apparent whole tract digestibility	0.65	0.65	0.65	0.65	0.052	0.57	0.72
ADFom							
Intake, g/d	130.1	130.3	130.3	130.1	2.22	0.98	0.51
Apparent digestibility in the forestomach	0.35	0.36	0.32	0.34	0.023	0.68	0.84
Apparent whole tract digestibility	0.50	0.49	0.49	0.52	0.009	0.35	0.64

TS0, TS400, TS600 and TS800 refer to the diets were supplemented with 0, 400, 600 and 800 mg TS/kg feed DM, respectively. L = linear effect; Q = quadratic effect.

Table 3
Effects of tea saponin levels on apparent digestibility in the small intestine of AA in goats.

	Treatments				P		
	TS0	TS400	TS600	TS800	SEM	L	Q
Arg	0.75	0.72	0.75	0.75	0.013	0.94	0.11
His	0.79	0.78	0.76	0.77	0.016	0.22	0.83
Ile	0.74	0.79	0.74	0.74	0.019	0.82	0.15
Leu	0.77	0.79	0.78	0.77	0.012	0.76	0.07
Lys	0.76	0.75	0.75	0.74	0.011	0.27	0.85
Phe	0.80	0.79	0.79	0.75	0.030	0.31	0.56
Met	0.78	0.69	0.72	0.68	0.029	0.07	0.53
Thr	0.74	0.69	0.73	0.72	0.025	0.75	0.40
Val	0.75	0.73	0.76	0.72	0.019	0.58	0.70
Ala	0.73	0.73	0.74	0.70	0.015	0.27	0.24
Gly	0.72	0.70	0.71	0.73	0.017	0.82	0.23
Tyr	0.75	0.68	0.75	0.76	0.021	0.50	0.61
Asp	0.66	0.72	0.67	0.62	0.026	0.34	0.07
Cys	0.69	0.71	0.72	0.72	0.016	0.16	0.52
Ser	0.71	0.71	0.69	0.70	0.006	0.27	0.63
Glu	0.71	0.75	0.76	0.74	0.027	0.37	0.42
TAA	0.74	0.73	0.75	0.73	0.007	0.40	0.28

TS0, TS400, TS600 and TS800 refer to the diets were supplemented with 0, 400, 600 and 800 mg TS/kg feed DM, respectively. L = linear effect; Q = quadratic effect. Arg = arginine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Phe = phenylalanine; Met = methionine; Thr = threonine; Val = valine; Ala = alanine; Gly = glycin; Tyr = tyrosine; Asp = aspartic acid; Cys = cysteine; Ser = serine; Glu = glutamate; TAA = total amino acid.

where Y_{ijkm} is the response, μ is the overall mean, Period_i is the effect of period, Goat_j is the effect of goat, Treatment_k is the effect of dietary TS supplementation levels, Hour_m is effect of hours postfeeding analyzed as repeated measures, The error (ε_{ijkm}) was assumed to be independent and N $(0, \sigma^2)$.

Statistical significance was declared if $P \leq 0.05$. Orthogonal polynomial contrasts were used to examine the linear and quadratic responses to increasing the supplementation level of TS in the diets. In the orthogonal polynomial analysis, coefficients were corrected for unequal spacing of treatments.

3. Results

Dietary TS levels did not affect the intake, apparent disappearance in the forestomach and apparent whole tract digestibility of DM, N, NDFom and ADFom, or apparent digestibility in the small intestine of DM and N (Table 2). The apparent digestibility in the small intestine of DM and N were 0.23 and 0.47, and the apparent whole tract digestibility of NDFom and ADFom were 0.54 and 0.50 respectively.

Table 4
Effect of tea saponin levels on ruminal fermentation parameters in goats.

	Treatments				P		
	TS0	TS400	TS600	TS800	SEM	L	Q
Ruminal pH	6.17	5.96	6.24	6.26	0.121	0.39	0.15
Ruminal NH_3-N (mg/dl)	22.82	13.35	16.22	15.98	0.916	0.20	0.25
Ruminal VFA (mmol/l)	40.6	44.3	36.4	34.7	2.86	0.47	0.22
Individual VFA (proportion of total VFA)							
Acetic acid	0.575	0.557	0.554	0.527	0.0128	0.33	0.51
Propionic acid	0.277	0.279	0.282	0.301	0.0104	0.14	0.33
Isobutyric acid	0.021	0.011	0.013	0.017	0.0022	0.14	0.16
Butyric acid	0.101	0.118	0.153	0.140	0.0093	0.16	0.55
Isovaleric acid	0.016	0.013	0.014	0.015	0.0013	0.50	0.17
Valeric acid	0.011	0.011	0.012	0.013	0.0009	0.16	0.66

TS0, TS400, TS600 and TS800 refer to the diets were supplemented with 0, 400, 600 and 800 mg TS/feed DM, respectively. VFA: total volatile fatty acids. L = linear effect; Q = quadratic effect.

Table 5
Effect of tea saponin levels on plasma parameters in goats.

	Treatment				P		
	TS0	TS400	TS600	TS800	SEM	L	Q
TG, mmol/L	0.28	0.25	0.20	0.22	0.017	<0.01	0.45
SOD, U/L	44.5	50.7	44.4	47.3	3.22	0.73	0.46
GSH-Px, μmol/L	392	399	408	372	23.9	0.73	0.37
MDA, nmol/L	4.12	3.59	3.28	3.77	0.532	0.49	0.44

TS0, TS400, TS600 and TS800 refer to the diets were supplemented with 0, 400, 600 and 800 mg TS/kg feed DM, respectively. TG = triacylglycerols; SOD = superoxidase dismutase; GSH-Px = glutathione peroxidase; MDA = malondialdehyde. L = linear effect; Q = quadratic effect.

There were no differences in the apparent digestibility in the small intestine of individual AA among treatments (Table 3). The average apparent digestibility in the small intestine of individual AA was ~0.74. There were no treatment effects on any rumen fermentation parameter (Table 4).

The plasma content of TG declined linearly ($P<0.01$) with increasing TS inclusion, but there were no differences in plasma SOD, GSH-Px and MDA (Table 5).

4. Discussion

4.1. Digestibility and ruminal fermentation

That supplementation of TS did not affect apparent whole tract digestibility of NDFom and ADFom might be due from a minor effect of dietary TS substances on Gram negative bacterium (Wina et al., 2005). The current study did not result in significant differences in apparent digestibility in the small intestine of total amino acids (TAA) among treatments.

Ruminal pH is an important index of rumen ecosystem function. The rumen pHs of 5.96–6.26 in this study were within the normal range of efficient rumen function (Yoon and Stern, 1996). That concentrations of acetate, propionate and butyrate were not changed when goats were fed diets supplemented with TS confirms that TS had minor effects on the pattern of rumen fermentation, just as Wang et al. (1998) and Hess et al. (2003) reported.

4.2. Plasma parameters

Shirai and Suzuki (2003) reported that the plasma TG content in their higher TS dose group was lower than that of the control group, which is consistent with our results which showed that the plasma TG content decreased with increased supplementation levels of TS.

The antioxidant activity of saponins has been investigated as saponins exert a protective action against free radical-induced tissue injury (Huong et al., 1998). Oxidative stress, caused as a result of physiological aerobic metabolism in mammals, can lead to the onset of diseases such as cancer, hypertension, diabetes and atherosclerosis (Zelko et al., 2002; Rosenblat et al., 2008). Thus markers of oxidative status were studied because they have all been implicated in the pathways which link oxidation to pathologic process. In general, endogenous antioxidants can be divided into three major groups with the first, enzymatic antioxidants, including superoxodase dismutase (SOD) and glutathione peroxidase (GSH-Px), which are the main form of intracellular antioxidant defense (Bernabucci et al., 2005). Indeed SOD is considered the first defence against pro-oxidants which convert superoxide to hydrogen peroxide; whereas erythrocyte GSH-Px converts H_2O_2 into less dangerous reduced forms (Halliwell and Chirico, 1993). As the plasma content of SOD was not affected by levels of TS, this indicates a better cellular defence against diseases such as cancer.

Halliwell and Chirico (1993) reported that plasma GSH-Px activity may be related to plasma lipid peroxide content, and Tüzün et al. (2002) considered plasma GSH-Px to be an indicator of oxidative stress, although the role of plasma GSH-Px as an antioxidant is unclear (Brigelius-Flohé, 1999). As the plasma GSH-Px content was not affected by TS supplementation, this indicates a high antioxidant status of the goats.

As an end product of lipid peroxidation, formation of malondialdehyde is accelerated by oxidative stress (Horie et al., 1997) and thus detection of MDA can reflect the level of oxygen free radicals and the extent of lipid peroxidation. Our results showing that the plasma MDA concentration was not affected by dietary TS supplementation indicates that TS could inhibit formation of MDA and reduce lipid peroxidation.

5. Conclusions

Results demonstrate that dietary supplementation of TS did not affect nutrient digestibility, ruminal fermentation or most plasma antioxidant parameters, but it did decrease the plasma TG concentration thereby increasing the antioxidant ability of the goats.

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References

AOAC, 1990. Official Methods of Analysis, 15th edition. Association of Official Analytical Chemists, Washington, DC, USA.

Bernabucci, U., Ronchi, B., Lacetera, N., Nardone, A., 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy Sci. 88, 2017–2026.

Brigelius-Flohé, R., 1999. Tissue-specific functions of individual glutathione peroxidase. Free Radic. Biol. Med. 27, 951–965.

Chaney, A.L., Marbach, E.P., 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8, 130–132.

Halliwell, B., Chirico, S., 1993. Lipid peroxidation: its mechanism, measurement, and significance. Am. J. Clin. Nutr. 57, 715S–725S.

Hess, H.D., Kreuzer, M., Diaz, T.E., Lascano, C.E., Carulla, J.E., Soliva, C.R., Machmuller, A., 2003. Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated rumen fluid. Anim. Feed Sci. Technol. 109, 79–94.

Horie, K., Miyata, T., Maeda, K., Miyata, S., Sugiyama, S., Sakai, H., van Ypersole, de Strihou, C., Monnier, V.M., Witztum, J.L., Kurokawa, K., 1997. Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. Implication for glycoxidative stress in the pathogenesis of diabetic nephropathy. J. Clin. Invest. 100, 2995–3004.

Hristov, A.N., McAllister, T.A., Van Herk, F.H., Cheng, K.J., Newbold, C.J., Cheeke, P.R., 1999. Effect of *Yucca schidigera* on ruminal fermentation and nutrient digestion in heifers. J. Anim. Sci. 77, 2554–2563.

Hu, W.L., Liu, J.X., Ye, J.A., Wu, Y.M., Guo, Y.Q., 2005. Effect of tea saponin on rumen fermentation *in vitro*. Anim. Feed Sci. Technol. 120, 333–339.

Hu, W.L., Liu, J.X., Wu, Y.M., Guo, Y.Q., Ye, J.A., 2006. Effects of tea saponins on in vitro ruminal fermentation and growth performance in growing Boer goat. Arch. Anim. Nutr. 60, 89–97.

Huong, N.T., Matsumoto, K., Kasai, R., Yamasaki, K., Watanabe, H., 1998. *In vitro* antioxidant activity of Vietnamese ginseng saponin and its components. Biol. Pharm. Bull. 21, 978–981.

Ikeda, I., Imasato, Y., Sasaki, E., Nakayama, M., Nagao, H., Takeo, T., Yayabe, F., Sugano, M., 1992. Tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats. Biochim. Biophys. Acta 1127, 141–146.

Lila, Z.A., Mohammed, N., Kanda, S., Kamada, T., Itabashi, H., 2003. Effect of sarsaponin on ruminal fermentation with particular reference to methane production in vitro. J. Dairy Sci. 86, 3330–3336.

Liu, J.X., Yuan, W.Z., Ye, J.A., Wu, Y.M., 2003. Effect of tea (*Camellia sineis*) saponin addition on rumen fermentation in vitro. Trop. Subtrop. Agroecosyst. 3, 561–564.

Lu, D.X., Zhang, P.Y., 1996. Scientific Technology of Feeding Goat. China Agriculture Press, Beijing, China [in Chinese], pp. 71–126.

Mason, V.C., Bech-Anderson, S., Rudemo, M., 1980. Hydrolysate preparation for amino acid determinations in feed constituents. 8. Studies of oxidation conditions for streamlined procedure. Z. Tierphysiol. Tierernährg. Futtermittelkd. 43, 146–164.

Rosenblat, M., Volkova, N., Coleman, R., Almagor, Y., Aviram, M., 2008. Antiatherogenicity of extra virgin olive oil and its enrichment with green tea polyphenols in the atherosclerotic apolipoprotein-E-deficient mice: enhanced macrophage cholesterol efflux. J. Nutr. Biochem. 19, 514–523.

SAS Institute, 1996. SAS/STAT® User's Guide (Release 6.11). SAS Institute Inc., Cary, NC, USA.

Schurch, A.F., Lloyd, L.E., Crampton, E.W., 1950. The use of chromic oxide as an index for determining the digestibility of a diet. J. Nutr. 41, 629–636.

Sgorlon, S., Stradaioli, G., Stefanon, B., Altimer, G., Della-Loggia, R., 2005. Dietary grape poliphenols modulate oxidative stress in ageing rabbits. In: Della Loggia, R. (Ed.), Proceedings of 16th Scientific Association of Animal Production (ASPA) Congress, Nat. Congr. Aspa, Torino, Italy. Ital. J. Anim. Sci. 4, 541–543.

Shirai, N., Suzuki, H., 2003. Effects of simultaneous docosahexaenoic acid and catechin intakes on the plasma and liver lipids in low- and high-fat diet fed mice. Nutr. Res. 23, 959–969.

Sun, Z.H., Tan, Z.L., Yao, J.H., Tang, Z.R., Shan, J.G., Hu, J.P., Tang, S.X., 2007. Effects of intra-duodenal limiting amino acids on immunoreaction, total DNA and RNA contents of skeletal muscles and jejunal mucous membrane for growing goat fed a mixture of maize stover and concentrate. Small Rum. Res. 69, 159–166.

Tan, Z.L., Lu, D.X., Hu, M., Niu, W.Y., Han, C.Y., Ren, X.P., Na, R., Lin, S.L., 2001. Effects of dietary nitrogen sources on fiber digestion and ruminal fluid characteristics in sheep fed wheat straw. Asian-Aust. J. Anim. Sci. 14, 1374–1382.

Tüzün, A., Erdil, A., Inal, V., Aydm, A., Bağci, S., Yesilova, Z., Sayal, A., Karaeren, N., Dağalp, K., 2002. Oxidative stress and antioxidant capacity in patient with inflammatory bowel disease. Clin. Biochem. 35, 569–572.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Symposium: carbohydrate methodology, metabolism and nutritional implications in dairy cattle. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Wang, Y.X., McAllister, T.A., Newbold, C.J., Rode, L.M., Cheeke, P.R., Cheng, K.J., 1998. Effect of *Yucca schidigera* extract on fermentation and degradation of steroidal saponins in the rumen simulation technique (RUSITEC). Anim. Feed Sci. Technol. 74, 143–153.

Wina, E., Muetzel, S., Hoffmann, E., Makkar, H.P.S., Becker, K., 2005. Saponins containing methanol extract of Sapindus rarak affect microbial fermentation, microbial activity and microbial community structure in vitro. Anim. Feed Sci. Technol. 121, 159–174.

Ye, J.A., 2001. Effects of saponins on production performance in Hu sheep. Feed Res. 6, 33 (in Chinese).

Yoon, I.K., Stern, M.D., 1996. Effect of *Saccharomyces cerevisiae* and *Aspergillus oryzae* cultures on ruminal fermentation in dairy cows. J. Dairy Sci. 79, 411–417.
Zelko, I.N., Mariani, T.J., Folz, R.J., 2002. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution and expression. Free Radical. BioMed 33, 337–349.
Zhang, H.F., Zhang, Z.Y., 1998. Animal Nutrition Parameters and Feeding Standard. China Agriculture Press, Beijing, China (in Chinese), pp. 22–92.
Zhong, R.Z., Tan, C.Y., Han, X.F., Tang, S.X., Tan, Z.L., Zeng, B., 2009. Effect of dietary tea catechins supplementation in goats on the quality of meat kept under refrigeration. Small Rum. Res. 87, 122–125.