*Article*

# Inclusion of Different Molecular Weight Condensed Tannin on Ruminal Fermentation and Milk Fatty Acid Profile of Dairy Goats

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**Abstract:** The aim of this study was to investigate the effect of condensed tannin (CTs) with differing molecular weight on their capacity to modify the fatty acid profile in milk. Twenty multiparous crossbred lactating dairy goats were assigned in a randomized complete block design (RCBD), and were subjected to receive the dietary treatments as followings; T1: control (with no CTs supplementation), T2: supplemented with mangosteen peel in a concentrate as a source of low molecular weight CTs at level of 3.0 %DM of CTs equivalent, T3: supplemented with the same diet with T2 but added with polyethylene glycol (PEG, as tannin inactivator) as the control of T2, and T4: supplemented with quebracho CT extract (UNITAN ATO, Buenos Aires, Argentina; 75-77 % tannins) in a concentrate as a source of high molecular weight CTs at level of 3.0 %DM of CTs equivalent, and T5) supplemented with the same diet with T4 but added with PEG as the control of T4. No significant change was detected for feed intake and nutrient digestibility indicate that CTs at level of 3.0 %DM of diet did not showed the

detrimental effect to feed intake and nutrient digestibility, however, ruminal fermentation parameters and milk yield and milk compositions did not affected by different source of CT inclusion.

**Keywords:** CTs molecular weight; ruminal fermentation; bio-hydrogenation; milk compositions; goat

## 1. Introduction

Utilization of tannins-containing plant materials in the tropical regions for ruminant feeding for improvement of animal performances and the shortage and rising costs of the conventional feed stuffs. Condensed tannins (CTs) are widely distributed in forages, shrubs, legumes, and also cereals and grains [1,2,3]. Although condensed tannins that presents in these plant materials resulting in adverse anti-nutritional effects to the animals which consumed them, feeding quality of those plant materials since these secondary metabolites have been reported to cause a variety of detrimental effects, such as decreased feed palatability, voluntary intake and nutrients digestibility [4,5,6]. However, in ruminant nutrition, condensed tannins have been recognized to be beneficial useful phyto-chemicals for rumen manipulation to increase rumen-undegradable protein supply to the small intestine, essential oil, and bloat prevention [7,8], anthelmintic property [9] and mitigating methane emission [10]. Currently, concerning of healthiness food for consumption have been greater considered. Ruminant food products particularly tissue lipids are recognised to be highly saturated in nature compared to non-ruminants, however, ruminant products also contain potentially health-promoting fatty acid includings conjugated linoleic acid (CLA), mainly *cis*-9, *trans*-11-CLA isomer (rumenic acid, RA), since they in many animal studies to contribute to cancer prevention, decreased atherosclerosis, improved immune response, and altered protein or energy metabolism

[11,12,13]. The CLA isomers can formed as the intermediates of biohydrogenation of polyunsaturated fatty acids including linolenic and linoleic acids [14] to the final product of this process, stearic acid [15]. In addition, rumenic acid can also be formed endogenously in the muscle or in the mammary gland from vaccenic acid (*trans*-11 C18:1, VA) [16] through the action of $\Delta^9$-desaturase enzyme [17] indicating that factors influencing the production of VA in the rumen are of interest in order to manipulate the biohydrogenation for increasing the CLA content in milk or meat of ruminant [18, 19]. Since many researches have been stated that there are two group of ruminal bacteria involved in ruminal biohydrogenation which have been categorized into Group A (have ability to hydrogenate polyunsaturated fatty acids to VA) and Group B (ability to hydrogenate polyunsaturated fatty acids through to SA) bacteria [20]. Nutritional strategies of supplementation of fish oil in ruminant diets have been very succesful in order to inhibit the final biohydrogenation step from VA to SA by its toxic effect on certain bacterial species [19], however, recently, the action of plant secondary compounds including essential oils, saponins and tannins which possess anti-microbial properties is greater attention [21, 10]. However, information pertaining to the effects of tannins on ruminal BH process and fatty acids composition of ruminant derived products is very limited, in paticularly using of difference source and structure of condensed tannins. Therefore, research emphasis on effect of condensed tannin with differing structure (particularly molecular weight) on ruminal BH and alteration of fatty acids composition of ruminant-derived food products would be further investigated.

The objective of this study was to evaluate the effect of inclusion of CTs sources with differing molecular weight in Sunflower oil-riched diet on voluntary feed intake, nutrients digestibility, ruminal fermentation and milk yield and milk fatty acid profile of lactating dairy goats. Therefore, strategy which can inhibit the group B bacteria and resulting in inhibit the final step of biohydrogenation process is greater interest.

## 2. Results

### *2.1 Chemical composition of experimental diets*

The dietary formula and chemical compositions of dietary treatments and some individual feeds used in this experiment is presented in Table 1. Concentrate feeds contained 18.5-18.6 % DM of crude protein, EE between 7.8-8.1 % DM and condensed tannins at 3.0 % DM. Concentrate feeds were offered to all goats as the sources of protein, energy and also unsaturated fatty acid. Table 2 shows the fatty acid compositions of experimental diets and individual feeds. Sunflower oil contains high proportion of polyunsaturated fatty acid (PUFA) (50 g/100 g FA) particularly C18:2n6c fatty acid (49.49.4 g/100 g FA). Although pangola hay contains high proportion of PUFA (12.34 and 18.11 g/100 g FA for C18:2n6c and C18:3n3 fatty acids, respectively), however, due to low content of fat, it seems to not be a good source of those fatty acids compared to other species of grass such as *Pennisetum purpureum*.

### *2.2 Feed intake, nutrients intake and nutrient digestibility*

Voluntary feed intake and intake of nutrients are presented in Table 3. Level of CTs at 3.0 % DM in concentrate feed seem to be high, however, this experiment was offered concentrate to experimental animal at 60 percent of total feed intake, therefore there were around 1.8 % of total DM feed intake. However, there were very low intake of feed contained CTs source such as mangosteen peel and quebracho extract and some animal did not accept the diet at the beginning of the experiment, therefore, we suggest a longer period for adaptation of animal to the CT-containing diet.

This study demonstrated that the diet contains CTs with both high or low molecular weight at 3.0 %DM (1.8 % of total feed) have no effect on voluntary feed intake ($p>0.05$). The amount of feed intakes was in normal range (2.8-3.0 %BW). Moreover, according to the results

of this study, molecular weight of CTs have no effected on nutrient intake and nutrient digestibility (Table 3).

*2.3 Intake of fatty acids*

Intake of dietary fatty acids of lactating dairy goats fed diet contained differing molecular weight of CTs are presented in Table 4. There was found that all goats from each treatment received similarly amount of PUFA and intake of total fatty acid (ranged from 17 to 24 g/d and 47 to 65 g/d, respectively).

*2.4 Ruminal fermentations*

Ruminal pH and volatile fatty acids concentration affected by inclusion of CTs of differing molecular weight are presented in Table 5. Molecular weight of CTs did not affected on ruminal fermentation. The pH values at 0 h post feeding of ruminal fluid did not significant different ($p > 0.05$), however, at 3-h post feeding, ruminal pH of goats fed diet contained CTs from mangosteen peel was significantly lower than others. Moreover, there were found that CTs with differing molecular weight did not affected on ruminal fermentation parameters such as concentration of volatile fatty acids.

*2.5 Milk yield and milk compositions*

Milk yield and milk compositions are presented in Table 6. Inclusion of CTs of differing molecular weight in the diet contained high level of sunflower oil did not effect on milk yield, milk compositions and milk fatty acids profile (Table 7, Table 8). Molecular weight of CTs did not affected on the milk fatty acid compositions. Milk from all goat's treatments. Stearic acid, the final product of biohydrogenation process in the rumen did not significant different among treatment and ranged between 9 – 14 g/100 g FA, indicated that inclusion of CTs with differing

molecular weight may not inhibit the growth of bacteria involved the final step of the biohydrogenation process.

## 3. Discussion

Many researches have been suggested that different sources (different structure) and levels of tannins effected on ruminal microorganisms and fermentation parameters. In particularly, inhibitory effect of tannins on ruminal methanogens and methane production have recently attention. Recently researches have been indicated that different sources (and molecular weights) of tannins has different effect on ruminal methane production [22] and methane production also decreased with increasing of level of condensed tannins [22,23].

Moreover, the effects of tannins on *B. fibrisolvens* have also been investigated by Jones *et al*. [24], who found *in vitro* that tannins from sainfoin leaves inhibit the growth and activity of *B. fibrisolvens* A38 by causing changes in the bacteria's morphology. This concept was confirmed by study of Huang *et al*. [22] who fractionated CTs from *Leuceana leucocephala* hybrid into five fractions by a size exclusion chromatography procedure, and determined the molecular weights and protein-binding affinities of the CT fractions, and indicated that, in general, CTs of higher molecular weight fractions have stronger protein-binding affinity than those of lower molecular weights, and in additionally, CT fractions with the highest MW had in the highest inhibition of $CH_4$ production, being 62% lower than the control [22] . In addition, Tan *et al*. [6] also reported linear reductions in total methanogens and total protozoa with increasing levels of CT.

Khiaosa-Ard *et al*. [25] reported that addition of CT (7.9 % of DM) inhibited the last step of linolenic acid (LNA) biohydrogenation in RUSITEC. This inhibition led to the accumulation of vaccenic acid (VA) in the feed residues but had no effect on the losses of LNA compared with the control treatment. Vasta et al. [26] also reported that the concentration of

VA increased while concentration of total conjugated linoleic acid (CLA) did not increase in *in vitro* ruminal fluid. Moreover, in *in vivo* studies, indicated that addition of quebracho tannins in the diet of sheep resulted in an increased concentrations of VA in the rumen and rumenic acid (RA) and poly unsaturated fatty acids (PUFA) in lamb [27, 28].

In addition, *in vivo* study by [28] found that the concentration of RA (C18:2 *c*9, *t*11) in goat kid groups supplemented with extract tannins of *T. chebula* at 1.06 and 3.18 g/kg BW were higher ($P \leq 0.05$) than in the control group. The concentration of VA was higher ($P \leq 0.01$) in group supplemented with 3.18 g tannins/kg BW compared to another groups, while the stearic acid (SA) concentration was lower ($P \leq 0.05$) in group supplemented with 3.18 g tannins/kg BW than control and supplemented with 1.06 g tannins/kg BW. However, there was found that supplementation of quebracho tannins to lambs at 95.7 g/kg DM did not affected on fatty acid profile of plasma [27]. Decreased saturated fatty acid (SFA) and increased unsaturated fatty acids (UFA) in *T. chebula* extract supplemented groups indicated an influence on rumen biohydrogenation and resultant higher absorption of the UFA as evident from the fatty acid profile of blood and muscle. It is also suggested from the present investigation that *T. chebula* extract affects rumen biohydrogenation as well as protects the UFA in plasma and tissues from lipid peroxidation (higher enzymatic and nonenzymatic antioxidative activity) which increases the animal product quality [28].

Rana *et al*. [28] reported that the RA concentration of *Longissimus dorsi* muscle was higher ($P \leq 0.01$) in goat fed tannins than in the control group, while total CLA concentration was significantly higher ($P \leq 0.01$) in group fed highest level of tannins. The SA content in muscle of kids of group supplemented with 3.18 g tannins/kg BW was significantly ($P \leq 0.05$) lower than in control group. However, study by [27] was found that supplementation of quebracho tannins to lambs at 95.7 g/kg DM did not affected on fatty acid profile of this muscle.

Although supplemented with tannins shown an affected to fatty acid composition of

ruminant meat, however, studies by Toral *et al*. [29,30] demonstrated that inclusion of tannins in diet not affected on milk fatty acid composition, and they were suggested that addition of quebracho tannins to a diet rich in linoleic acid did not prove useful to beneficially modify milk FA composition, especially over the long term, and structural and chemical dissimilarities between HT and CT may offer an explanation for differences in their biological effects and, therefore, results obtained using a particular type of tannins cannot be applied to others. A key issue when using plant extracts as feed additives is dosage [29]. Most doses of tannins evaluated in the studies quoted above ranged from 4.5 g/kg DM [31] to 44.5 g/kg DM [32].

This study aimed to investigate the effect of inclusion of different molecular weight of condensed tannins on ruminal fermentation and milk fatty acid composition in dairy goat, with the hypothesis that, higher molecular weight of CTs had stronger inhibitory effects on ruminal methane production and inhibit the final step of ruminal biohydrogenation process which could results in higher unsaturated fatty acid and lower saturated fatty acid concentration in goat milk. For these objectives of study, the study was carried out comprising 3 experiments. The first experiment was conducted to determine the component of phenolic compounds such as total phenol, total tannins and condensed tannins, and molecular weight and protein-binding ability of condensed tannins in the selected tropical plant materials and commercial condensed tannins extract [1, 3,33]. The previous experiment was conducted to investigate the effect of different molecular weight (higher and lower) of condensed tannins on *in vitro* gas production kinetics, methane production and fermentation parameters [2]. This experiment was carried out to determine the effect of inclusion of condensed tannins sources with differing molecular weight on feed intake and nutrient digestibility, and milk yield and milk fatty acid composition of dairy goats.

Selected tropical plant materials and tannins extract with potential use as the sources of condensed tannins (CTs) for ruminant feeding, including, leucaena, cassava, Siamese neem

leaves, mangosteen peel and quebracho extract. Among of those plant species leaves, Siamese neem contained highest concentration of CTs and highest molecular weight. Furthermore, protein-binding ability, a biological property of CTs which is the major factor influent on CTs activities, had relative high with higher molecular weight of CTs.

Supplementation of CTs of higher MW from leaves of Siamese neem significantly inhibited *in vitro* total gas and methane productions while supplementation of the low MW condensed tannins from leaves of leucaena had no effect, except for total gas production at the highest (6 mg/100 mg DM) level of supplementation. Moreover, Siamese neem leaves had stronger effect ($p < 0.001$) on *in vitro* volatile fatty acids production.

Inclusion of CTs sources with represents the higher and lower molecular weight (quebracho extract and mangosteen peel powder, respectively) at 3%DM of CTs equivalent have no detrimental effect to feed intake and nutrient digestibility, similarly, ruminal fermentation parameters and milk yield and milk compositions did not affect by different source of CT inclusion.

Currently, concerning of healthiness food for consumption have been greater considered. To produce healthy benificial foods (milk and meat) from ruminats, nutritional strategies in order to manipulate rumen fermentation which results in improving the animal products has greater interested. Utilization of plants containing tannins as the source of natural bioactive agent for ruminant feeding is a part of most effective strategies. However, there were found that supplementation of tannins-containing plants to ruminant animal had a varied results. Therefore, more understanding of tannins characteristic of these plants could be useful for this strategy application. The novelty of this work is that we explore a molecular weight characteristic of condensed tannins of tropical avialable plant species which has potential use as a source of tannins in ruminant feeding. However, further researchs involving selective inhibitory effect to some baceria involved in ruminal biohydrogenation, dose of using of

various sources with differing molecular weight of condensed tannins and molecular-based on experiment in rumen microbes should be investigate.

## 4. Materials and methods

### *4.1 Experimental animals, design, and diet*

This experiment was conducted according to principles and guidelines approved by the Animal Care and Use Committee of Suranaree University of Technology, Nakhon Ratchasima, Thailand.

Twenty multiparous crossbred-Saanen lactating goats in early lactation period (30 ± 9.5 day in milk) with 0.83 ± 0.32 kg per day of milk yield and 41.2 ± 7.2 kg of body weight were subjected to use in this experiment. All goats were housed in individual pens which able to free access to mineral block and water. All goats were assigned to a randomized complete block design (RCBD) with 4 blocks (replicates) per treatment. The experimental period was divided into a 14-days adaptation period and a 21-days of experimental period. The lactating goats were subjected to hand milking twice a day and fed a pangola hay *ad libitum* as the soul roughage source, and supplemented with the following dietary concentrate treatments twice a day at the milking times (7.00 a.m. and 16.00 p.m.), the dietary treatments composed T1) control (with no CTs supplementation), T2) supplemented with mangosteen peel in a concentrate as a source of low molecular weight CTs at level of 3.0 %DM of CTs equivalent, T3) supplemented with the same diet with T2 but added with polyethylene glycol (PEG, as tannin inactivator) as the control of T2, T4) supplemented with quebracho CT extract (UNITAN ATO, Buenos Aires, Argentina; 75-77 % tannins) in a concentrate as a source of high molecular weight CTs at level of 3.0 %DM of CTs equivalent, and T5) supplemented with the same diet with T4 but added with PEG as the control of T4.

All concentrate treatments were calculated to contain crude protein (CP) at 18.5 % DM

by using of the 16 and 21 %CP commercial pelleted concentrates (produced by Suranaree University of Technology Farm, SUT Farm) and molasses as the feed ingredients (Table 1). All commercial concentrates used as the feed ingredient were ground in order to make diet mixed well prior to mix in the mixtures. Sunflower oil was added to the ration at 5 % DM daily before feeding, to avoid the rancidity of the diet.

*4.2 Sample collection, measurements and analysis*

All goats were weighed at the beginning and end of the experiment to calculate feed intake. All feed and feed residue samples were collected weekly for chemical analysis. Feeds offered and remained were recorded daily during the last 14 days of experimental period to calculate feed intake and nutrients digestibility measurement. Feces from all goats were sampled about 10 % at last two weeks of the experimental period. All feed samples of each treatments were pooled at the end of experiment and divided into two parts, one for dry mater (DM) measurement by using hot air oven at 100 °C, 48 h, and another part was dried at 60 °C prior to grind pass through a 1.0 mm screen and subjected to proximate analysis. Feed and feces samples were analyzed for DM, crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), ADF, and acid insoluble ash (AIA). All chemical compositions were expressed on DM basis.

In addition, feeds and sunflower oil samples were analysis for fatty acid compositions. Fatty acids in feed samples were extracted using a method of Folch *et al*. [34] and Metcalfe *et al.* [35] with minor modified. In brief, Fifteen gram of each sample was homogenized with 90 mL of chloroform-methanol (2:1) for 2 min. Then, further homogenized for 2 min with 30 mL of deionized water prior to adding of 5 mL of 0.58% NaCl. The fatty acid methyl esters layer was moved and placed in a screw-cap test tube and stored at -20 °C until further methylation. To prepare the fatty acid sample for determine fatty acid composition, fatty acid methyl esters

(FAME) were prepared by the method described by [36]. Briefly, transferred approximately 30 mg of the extracted oil into a screw-cap tube fitted with a Teflon-lined. Adds 1.5 mL of 0.5 M NaOH in methanol into the tube, dried with $N_2$ about 30 sec and immediately capped the cap. Heat the sample at 100 °C for 2 min with occasional shaking and then cooled the sample at room temperature. After that, 1.0 mL of C17:0 internal standard (1.0 mg/mL of trimethyl pentane) and 2.0 mL of 14 % boron trifluoride ($BF_3$) in methanol were added, dry with N2 for 30 sec and immediately capped. Heated sample mixture at 100 °C for 30 min with occasional shaking and cooled at room temperature. Add 5.0 mL of trimethyl pentane, shake and then added with 5.0 mL of saturated NaCl and shake. The upper clear part was collected and transfer to the 1.0 mL vial and capped, FAME samples were kept at -20 °C until further analysis for fatty acid profile using Gas Chromatography (GC) [37].

Milk samples were determined for compositions including fat, protein, lactose, total solids (TS), and solid-not-fat (SNF) by MilkoScan FT2 infrared automatic analyser (FOSS, Hillerod, Denmark).

### *4.3 Statistical analysis*

All data were subjected to one-way analysis of variance (ANOVA) procedure. Means were separated using Duncan's procedure where differences were $P<0.05$ among treatments. All data were analyzed using SAS software [38].

### *4.4 Location of the study*

The study was conducted at the Center for Scientific and Technological Equipment Building 1 and 10, Suranaree University of Technology, Nakhon Ratchasima, Thailand.

## 5. Conclusion

Based on the current results, inclusion of CTs with differing molecular weight in the diet rich-in sunflower oil did not affected on the fatty acid compositions of milk. However, although inclusion of CTs from both mangosteen peel and quebracho extract not altered the fatty acid profile, in the other hand, further study focus on the molecular weight and dose of CTs used would be investigated in particular their effect on bacteria involved in this process.

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**Table 1** Feed ingredient, chemical and major fatty acid composition of dietary treatments

| Items | Experimental diets and feed stuff | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2,T3[/2] | T4,T5[3/] | Pangola hay | MSP[4/] | QB[5/] |
| *Ingredient composition (% DM)* | | | | | | |
| SUT concentrate (16%CP)[/1] | 26.0 | 0.0 | 9.0 | - | - | - |
| SUT concentrate (21%CP)[/1] | 68.0 | 73.0 | 80.5 | - | - | - |
| Dried-MSP powder | - | 21 | - | - | - | - |
| QB extract powder | - | - | 4.5 | - | - | - |
| Molasses | 1 | 1 | 1 | - | - | - |
| Sunflower oil | 5 | 5 | 5 | - | - | - |
| *Chemical composition (% DM)*[/6] | | | | | | |
| DM (%) | 92.49 | 92.45 | 92.54 | 88.82 | 90.57 | 91.28 |
| OM | 92.47 | 93.12 | 92.37 | 94.40 | 96.15 | 91.71 |
| Ash | 7.53 | 6.88 | 7.63 | 5.6 | 3.85 | 8.29 |
| CP | 18.64 | 18.59 | 18.53 | 5.13 | 14.89 | 1.36 |
| EE | 8.11 | 7.84 | 8.05 | 1.51 | n.d. | n.d. |
| NDF | 42.45 | 48.36 | 42.21 | 74.38 | n.d. | n.d. |
| ADF | 23.56 | 31.62 | 21.65 | 41.16 | n.d. | n.d. |
| AIA | 1.06 | 1.09 | 1.10 | 1.95 | n.d. | n.d. |
| Condensed tannin | - | 3.0 | 3.0 | - | 14.4 | 67.8 |

[1/] Commercial concentrate pellet feed purchased from Suranaree University of Technology (SUT) farm.

[2/] T2 and T3 diets contains CTs form mangosteen peel as the source of low molecular weight CTs.

[3/] T4 and T5 diets contains CTs form quebracho extract as the source of high molecular weight CTs.

[4/] Mangosteen peel powder as the source of low molecular CTs.

[5/]Quebracho condensed tannins extract (UNITAN ATO, Buenos Aires, Argentina).

[6/]DM= dry matter; OM= organic matter; CP= crude protein, EE= ether extract, NDF= neutral-detergent fiber; ADF= acid-detergent fiber; AIA= acid insoluble ash.

n.d.= not determined.

**Table 2** Fatty acid compositions of experimental diets and some individual feeds.

| Items | Experimental diets and some individual feeds | | | | |
|---|---|---|---|---|---|
| | **T1** | **T2,T3** | **T4,T5** | **SFO** | **Pangola hay** |
| Fatty acid composition (g/100 g FA) | | | | | |
| C12 | 13.14 | 11.86 | 12.74 | 0.00 | 2.15 |
| C14 | 2.25 | 2.03 | 2.18 | 0.00 | 2.02 |
| C14:1 | 0.40 | 0.36 | 0.38 | 0.00 | 2.27 |
| C16 | 10.36 | 9.94 | 10.23 | 6.03 | 11.48 |
| C16:1 | 0.36 | 0.32 | 0.35 | 0.00 | 1.88 |
| C18 | 3.05 | 3.07 | 3.05 | 3.23 | 2.24 |
| C18:1n9c | 30.85 | 31.70 | 31.11 | 39.60 | 5.17 |
| C18:2n6c | 31.73 | 33.46 | 32.27 | 49.44 | 12.34 |
| C20 | 0.28 | 0.28 | 0.28 | 0.25 | 2.23 |
| C18:3n6 | 0.25 | 0.23 | 0.24 | 0.00 | 4.00 |
| C20:1 | 0.37 | 0.35 | 0.36 | 0.21 | 2.44 |
| C18:3n3 | 0.49 | 0.49 | 0.49 | 0.54 | 18.11 |
| C20:2 | 0.33 | 0.30 | 0.32 | 0.00 | 3.89 |
| C22:0 | 0.62 | 0.63 | 0.62 | 0.70 | 2.27 |
| C20:3 n6 | 0.32 | 0.29 | 0.31 | 0.00 | 3.01 |
| C22:1n9 | 0.24 | 0.22 | 0.23 | 0.00 | 0.08 |
| C20:3n3 | 0.18 | 0.16 | 0.17 | 0.00 | 1.94 |
| C20:4n6 | 0.19 | 0.17 | 0.18 | 0.00 | 1.82 |
| SFA[1/] | 29.7 | 27.81 | 29.1 | 10.21 | 22.39 |
| MUFA[2/] | 32.22 | 32.95 | 32.43 | 39.81 | 11.84 |
| PUFA[3/] | 33.49 | 35.1 | 33.98 | 49.98 | 45.11 |
| PUFA : SFA | 1.13 | 1.26 | 1.17 | 4.90 | 2.01 |

[1/]SFA = sum of saturated fatty acids (C12:0-C14:0, C16:0, C18:0: C20:0, C22:0).

[2/]MUFA = sum of monounsaturated fatty acid (C14:1, C16:1, C18:1n9, C20:1, C22:1n9).

[3/]PUFA = sum of polyunsaturated fatty acid (C18:2n6, C18:3n6, C18:3n3, C18:4n3, C20:2, C20:3n6, C20:3n3, C20:4n6).

**Table 3** Effects of inclusion of CTs of differing molecular weight on voluntary feed intake, nutrient intake and digestibility in dairy goat.

| Items | Treatments | | | | | P-value |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| Feed intake (kg DM/h/d) | | | | | | |
| *Concentrate* | 0.6309 | 0.5963 | 0.7271 | 0.6867 | 0.4732 | 0.1922 |
| *Pangola hay* | 0.5075 | 0.6127 | 0.5884 | 0.4752 | 0.6129 | 0.7317 |
| *Total* | 1.1384 | 1.2090 | 1.3156 | 1.1619 | 1.0861 | 0.5659 |
| Feed intake (% BW) | | | | | | |
| *Concentrate* | 1.5699 | 1.4220 | 1.7159 | 1.7583 | 1.2836 | 0.2190 |
| *Pangola hay* | 1.2913 | 1.4260 | 1.2988 | 1.2150 | 1.6616 | 0.2226 |
| *Total* | 2.8612 | 2.8480 | 3.0147 | 2.9732 | 2.9452 | 0.4270 |
| Nutrients intake (kg DM/d) | | | | | | |
| *Organic matter* | 1.0625 | 1.1336 | 1.2326 | 1.0829 | 1.0157 | 0.5511 |
| *Crude protein* | 0.14363 | 0.14228 | 0.16536 | 0.15162 | 0.11913 | 0.2202 |
| *EE* | 0.059 | 0.056001 | 0.065893 | 0.062453 | 0.047350 | 0.2316 |
| *NDF* | 0.64531 | 0.74407 | 0.78932 | 0.64329 | 0.65560 | 0.4307 |
| *ADF* | 0.35754 | 0.44073 | 0.47212 | 0.34425 | 0.35471 | 0.1024 |
| Nutrients digestibility (% of DM) | | | | | | |
| *Organic matter* | 75.854 | 73.468 | 71.318 | 73.477 | 72.252 | 0.7517 |
| *Crude protein* | 74.935 | 74.705 | 73.736 | 75.219 | 75.670 | 0.9851 |
| *NDF* | 68.979 | 66.526 | 66.834 | 66.333 | 69.251 | 0.9067 |
| *ADF* | 60.964 | 62.178 | 61.008 | 58.856 | 58.748 | 0.9568 |

T1) control (with no CTs supplementation), T2) supplemented with mangosteen peel in a concentrate as a source of low molecular weight CTs at level of 3.0 %DM of CTs equivalent, T3) supplemented with the same diet with T2 but added with polyethylene glycol (PEG, as tannin inactivator) as the control of T2, T4) supplemented with quebracho CT extract (UNITAN ATO, Buenos Aires, Argentina; 75-77 % tannins) in a concentrate as a source of high molecular weight CTs at level of 3.0 %DM of CTs equivalent, and T5) supplemented with the same diet with T4 but added with PEG as the control of T4.

**Table 4** Intake of major fatty acids.

| Items | Treatments | | | | | P-value |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| Intake of fatty acids (g/d) | | | | | | |
| *C12* | 6.886 | 5.742 | 6.950 | 7.197 | 5.053 | 0.2089 |
| *C14* | 1.304 | 1.134 | 1.335 | 1.349 | 1.017 | 0.2491 |
| *C16* | 6.182 | 5.709 | 6.686 | 6.480 | 4.960 | 0.2613 |
| *C18* | 1.731 | 1.641 | 1.947 | 1.849 | 1.371 | 0.2166 |
| *C18:1n9c* | 16.181 | 15.299 | 18.532 | 17.570 | 12.331 | 0.1977 |
| *C18:2n6c* | 17.183 | 16.784 | 20.171 | 18.722 | 13.435 | 0.1843 |
| *C20* | 0.316 | 0.337 | 0.357 | 0.316 | 0.314 | 0.7975 |
| *C18:3n6* | 0.434 | 0.475 | 0.484 | 0.421 | 0.462 | 0.8866 |
| *C20:1* | 0.376 | 0.392 | 0.419 | 0.377 | 0.365 | 0.8204 |
| *C18:3n3* | 1.638 | 1.907 | 1.891 | 1.571 | 1.863 | 0.7832 |
| *C20:2* | 0.469 | 0.501 | 0.518 | 0.458 | 0.484 | 0.9134 |
| *C22:0* | 0.491 | 0.504 | 0.560 | 0.507 | 0.448 | 0.4471 |
| *C20:3 n6* | 0.394 | 0.414 | 0.432 | 0.387 | 0.397 | 0.9262 |
| *C22:1n9* | 0.128 | 0.108 | 0.130 | 0.134 | 0.096 | 0.2900 |
| *C20:3n3* | 0.238 | 0.253 | 0.263 | 0.233 | 0.244 | 0.9146 |
| *C20:4n6* | 0.235 | 0.247 | 0.258 | 0.231 | 0.237 | 0.9324 |
| *SFA* | 16.91 | 15.07 | 17.84 | 17.70 | 13.16 | 0.2700 |
| *MUFA* | 16.69 | 15.80 | 19.08 | 18.08 | 12.79 | 0.2046 |
| *PUFA* | 20.59 | 20.58 | 24.02 | 22.02 | 17.12 | 0.2075 |
| *Total FA* | 54.19 | 51.45 | 60.93 | 57.80 | 43.08 | 0.2315 |

[1/]SFA = sum of saturated fatty acids (C12:0-C14:0, C16:0, C18:0: C20:0, C22:0).
[2/]MUFA = sum of monounsaturated fatty acid (C14:1, C16:1, , C18:1n9, C20:1, C22:1n9).
[3/]PUFA = sum of polyunsaturated fatty acid (C18:2n6, C18:3n6, C18:3n3, C18:4n3, C20:2, C20:3n6, C20:3n3, C20:4n6). T1) control (with no CTs supplementation), T2) supplemented with mangosteen peel in a concentrate as a source of low molecular weight CTs at level of 3.0 %DM of CTs equivalent, T3) supplemented with the same diet with T2 but added with polyethylene glycol (PEG, as tannin inactivator) as the control of T2, T4) supplemented with quebracho CT extract (UNITAN ATO, Buenos Aires, Argentina; 75-77 % tannins) in a concentrate as a source of high molecular weight CTs at level of 3.0 %DM of CTs equivalent, and T5) supplemented with the same diet with T4 but added with PEG as the control of T4.

**Table 5** Effects of inclusion of CTs of differing molecular weight on ruminal pH and volatile fatty acids concentration in dairy goat.

| Items | Treatments | | | | | P-value |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| Ruminal pH | | | | | | |
| - *0-h post feeding* | 7.12 | 7.07 | 6.95 | 7.03 | 7.05 | 0.1031 |
| - *3-h post feeding* | 6.79a | 6.71b | 6.76ab | 6.83a | 6.80a | 0.0330 |
| Ruminal VFAs | | | | | | |
| - *0-h post feeding* | | | | | | |
| Total VFAs (mM) | 37.57 | 40.92 | 47.46 | 47.60 | 43.69 | 0.0540 |
| Acetic acid (%) | 67.39 | 68.18 | 67.59 | 69.07 | 68.81 | 0.8195 |
| Propionic acid (%) | 21.38 | 20.96 | 21.86 | 19.49 | 20.49 | 0.4310 |
| Butyric acid (%) | 11.24 | 10.86 | 10.55 | 11.44 | 10.69 | 0.5883 |
| Acetic : Propionic | 3.31 | 3.39 | 3.18 | 3.59 | 3.41 | 0.6158 |
| *-3-h post feeding* | | | | | | |
| Total VFAs (mM) | 52.153 | 47.698 | 49.765 | 53.715 | 51.858 | 0.7026 |
| Acetic acid (%) | 65.653 | 67.805 | 67.645 | 69.985 | 66.313 | 0.6141 |
| Propionic acid (%) | 23.595 | 21.940 | 22.575 | 19.090 | 23.388 | 0.4736 |
| Butyric acid (%) | 10.7575 | 10.2525 | 9.7750 | 10.9275 | 10.3025 | 0.5553 |
| Acetic : Propionic | 2.9850 | 3.1925 | 3.0875 | 3.6825 | 2.9500 | 0.4921 |

T1) control (with no CTs supplementation), T2) supplemented with mangosteen peel in a concentrate as a source of low molecular weight CTs at level of 3.0 %DM of CTs equivalent, T3) supplemented with the same diet with T2 but added with polyethylene glycol (PEG, as tannin inactivator) as the control of T2, T4) supplemented with quebracho CT extract (UNITAN ATO, Buenos Aires, Argentina; 75-77 % tannins) in a concentrate as a source of high molecular weight CTs at level of 3.0 %DM of CTs equivalent, and T5) supplemented with the same diet with T4 but added with PEG as the control of T4.

**Table 6** Effects of inclusion of CTs of differing molecular weight on milk yield and milk composition in dairy goat.

| Items | Treatments | | | | | P-value |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| Milk yield (kg/d) | 0.96a | 0.80b | 1.03a | 1.00a | 0.52 | 0.0261 |
| Milk yield[1/] (kg/d) | 0.94 | 0.91 | 0.84 | 0.75 | 0.87 | 0.0261 |
| *P-value* (covariance analysis) | | | | | | |
| *T1* | * | 0.5178 | 0.0651 | 0.0025 | 0.2009 | |
| *T2* | 0.5178 | * | 0.2070 | 0.0100 | 0.4627 | |
| *T3* | 0.0651 | 0.2070 | * | 0.0812 | 0.5982 | |
| *T4* | 0.0025 | 0.0100 | 0.0812 | * | 0.0550 | |
| *T5* | 0.2009 | 0.4627 | 0.5982 | 0.0550 | * | |
| Milk composition (%) | | | | | | |
| *fat* | 3.89 | 3.76 | 3.88 | 3.79 | 3.90 | 0.1272 |
| *protein* | 3.30a | 3.31a | 3.22b | 3.23b | 3.26b | 0.0010 |
| *Lactose* | 4.67 | 4.74 | 4.65 | 4.66 | 4.66 | 0.1241 |
| *Total solids* | 12.52 | 12.45 | 12.45 | 12.38 | 12.53 | 0.7851 |
| *Solid-not-fat* | 8.80 | 8.76 | 8.70 | 8.68 | 8.75 | 0.9508 |

[1/]Yield adjusted (covariance analysis)

T1) control (with no CTs supplementation), T2) supplemented with mangosteen peel in a concentrate as a source of low molecular weight CTs at level of 3.0 %DM of CTs equivalent, T3) supplemented with the same diet with T2 but added with polyethylene glycol (PEG, as tannin inactivator) as the control of T2, T4) supplemented with quebracho CT extract (UNITAN ATO, Buenos Aires, Argentina; 75-77 % tannins) in a concentrate as a source of high molecular weight CTs at level of 3.0 %DM of CTs equivalent, and T5) supplemented with the same diet with T4 but added with PEG as the control of T4.

**Table 7** Effects of inclusion of CTs of differing molecular weight on milk fatty acid composition in dairy goat.

| Items | Treatments | | | | | P-value |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| Fatty acids (g/100 g FA) | | | | | | |
| *C4* | 1.51 | 1.45 | 1.24 | 1.48 | 0.94 | 0.0927 |
| *C6* | 2.01 | 2.16 | 1.92 | 2.18 | 1.67 | 0.0611 |
| *C8* | 2.24 | 2.63 | 2.39 | 2.64 | 2.30 | 0.0709 |
| *C10* | 7.17 | 8.15 | 7.71 | 8.51 | 8.31 | 0.2032 |
| *C11* | 0.19 | 0.17 | 0.17 | 0.13 | 0.14 | 0.9221 |
| *C12* | 5.35 | 6.98 | 6.27 | 5.60 | 6.78 | 0.0706 |
| *C13* | 0.02 | 0.00 | 0.03 | 0.00 | 0.00 | 0.6085 |
| *C14* | 10.76 | 9.73 | 9.18 | 10.54 | 10.81 | 0.0873 |
| *C14:1* | 0.38 | 0.26 | 0.37 | 0.25 | 0.34 | 0.4106 |
| *C15* | 1.11 | 0.94 | 1.04 | 0.76 | 0.88 | 0.0574 |
| *C15:1* | 0.16 | 0.04 | 0.07 | 0.14 | 0.04 | 0.2238 |
| *C16* | 30.31 | 24.29 | 24.93 | 24.90 | 25.21 | 0.1281 |
| *C16:1* | 0.56 | 0.37 | 0.44 | 0.64 | 0.79 | 0.8519 |
| *C17:1* | 0.22 | 0.11 | 0.11 | 0.17 | 0.11 | 0.5313 |
| *C18* | 9.06 | 12.86 | 12.61 | 13.77 | 10.58 | 0.4766 |
| *C18:1n9c* | 2.01 | 2.61 | 3.57 | 2.24 | 2.42 | 0.7245 |
| *C18:1n9t* | 19.183 | 18.27 | 20.56 | 20.53 | 20.915 | 0.6538 |
| *C18:2n6t* | 0.26b | 0.25b | 0.34ab | 0.33ab | 0.443a | 0.0340 |
| *C18:2n6c* | 4.13 | 5.35 | 4.29 | 3.4750 | 5.0050 | 0.1983 |
| *C20* | 0.32 | 0.29 | 0.28 | 0.19 | 0.31 | 0.2006 |
| *C18:3n6* | 4.13 | 5.35 | 4.30 | 3.47 | 5.01 | 0.5309 |
| *C20:1* | 0.07 | 0.04 | 0.00 | 0.00 | 0.05 | 0.2435 |
| *C20:2* | 0.22 | 0.49 | 0.62 | 0.22 | 0.59 | 0.2331 |
| *C22:0* | 0.15 | 0.13 | 0.05 | 0.04 | 0.00 | 0.0589 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *C20:3 n6* | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.0640 |
| *SFA* | 72.53 | 72.08 | 69.58 | 71.96 | 69.25 | 0.5149 |
| *MUFA* | 22.59 | 21.71 | 25.12 | 23.97 | 24.67 | 0.6017 |
| *PUFA* | 4.89 | 6.22 | 5.30 | 4.07 | 6.08 | 0.2067 |
| *PUFA : SFA* | 0.07 | 0.09 | 0.08 | 0.06 | 0.09 | 0.1976 |

[1/]SFA = sum of saturated fatty acids (C4:0-C14:0, C15:0, C16:0, C18:0: C20:0, C21:0, C22:0).

[2/]MUFA = sum of monounsaturated fatty acid (C14:1, C15:1, C16:1, C17:1, C18:1n9, C20:1, C22:1n9).

[3/]PUFA = sum of polyunsaturated fatty acid (C18:2n6, C18:3n6, C18:3n3, C18:4n3, C20:2, C20:3n6, C20:3n3, C20:4n6). T1) control (with no CTs supplementation), T2) supplemented with mangosteen peel in a concentrate as a source of low molecular weight CTs at level of 3.0 %DM of CTs equivalent, T3) supplemented with the same diet with T2 but added with polyethylene glycol (PEG, as tannin inactivator) as the control of T2, T4) supplemented with quebracho CT extract (UNITAN ATO, Buenos Aires, Argentina; 75-77 % tannins) in a concentrate as a source of high molecular weight CTs at level of 3.0 %DM of CTs equivalent, and T5) supplemented with the same diet with T4 but added with PEG as the control of T4.

**Table 8** Effects of inclusion of CTs of differing molecular weight on milk fatty acid yield in dairy goat.

| Items | Treatments | | | | | P-value |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| Yield of milk fatty acids (g/d) | | | | | | |
| *C4* | 0.53 | 0.42 | 0.44 | 0.52 | 0.18 | 0.2196 |
| *C6* | 0.69 | 0.61 | 0.74 | 0.77 | 0.32 | 0.3082 |
| *C8* | 0.77 | 0.73 | 0.97 | 0.93 | 0.43 | 0.3972 |
| *C10* | 2.47 | 2.26 | 3.11 | 3.02 | 1.57 | 0.4682 |
| *C11* | 0.06 | 0.04 | 0.06 | 0.04 | 0.03 | 0.6859 |
| *C12* | 1.77 | 1.93 | 2.43 | 1.99 | 1.30 | 0.5425 |
| *C13* | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.4895 |
| *C14* | 3.61 | 2.70 | 3.32 | 3.72 | 2.06 | 0.3210 |
| *C14:1* | 0.13 | 0.07 | 0.16 | 0.11 | 0.07 | 0.3469 |
| *C15* | 0.38 | 0.26 | 0.38 | 0.27 | 0.17 | 0.1961 |
| *C15:1* | 0.05 | 0.02 | 0.03 | 0.04 | 0.01 | 0.5569 |
| *C16* | 10.02 | 6.71 | 8.77 | 8.74 | 4.74 | 0.1590 |
| *C16:1* | 0.22 | 0.10 | 0.14 | 0.22 | 0.16 | 0.8499 |
| *C17:1* | 0.08 | 0.03 | 0.02 | 0.05 | 0.02 | 0.2204 |
| *C18* | 3.85 | 3.77 | 5.01 | 4.95 | 2.03 | 0.4338 |
| *C18:1n9c* | 0.76 | 0.82 | 1.59 | 0.91 | 0.49 | 0.5495 |
| *C18:1n9t* | 6.99 | 5.06 | 7.24 | 7.18 | 4.00 | 0.2645 |
| *C18:2n6t* | 0.10 | 0.07 | 0.12 | 0.11 | 0.08 | 0.4542 |
| *C18:2n6c* | 1.30 | 1.44 | 1.81 | 1.19 | 0.94 | 0.6379 |
| *C20* | 0.11 | 0.08 | 0.10 | 0.06 | 0.06 | 0.4625 |
| *C18:3n6* | 0.07 | 0.03 | 0.03 | 0.01 | 0.01 | 0.3336 |
| *C20:1* | 0.02 | 0.01 | 0.00 | 0.00 | 0.01 | 0.4860 |
| *C20:2* | 0.09 | 0.12 | 0.25 | 0.06 | 0.11 | 0.3348 |
| *C22:0* | 0.05 | 0.03 | 0.01 | 0.01 | 0.00 | 0.0499 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *C20:3 n6* | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.1024 |
| *SFA* | 25.01 | 20.18 | 25.98 | 25.43 | 13.14 | 0.3473 |
| *MUFA* | 8.25 | 6.12 | 9.18 | 8.51 | 4.75 | 0.3596 |
| *PUFA* | 1.57 | 1.65 | 2.21 | 1.37 | 1.14 | 0.5829 |
| *PUFA : SFA* | 0.07 | 0.09 | 0.08 | 0.06 | 0.09 | 0.1976 |

[1/]SFA = sum of saturated fatty acids (C4:0-C14:0, C15:0, C16:0, C18:0: C20:0, C21:0, C22:0, C23:0).

[2/]MUFA = sum of monounsaturated fatty acid (C14:1, C15:1, C16:1, C17:1, C18:1n9, C20:1, C22:1n9).

[3/]PUFA = sum of polyunsaturated fatty acid (C18:2n6, C18:3n6, C18:3n3, C18:4n3, C20:2, C20:3n6, C20:3n3, C20:4n6). T1) control (with no CTs supplementation), T2) supplemented with mangosteen peel in a concentrate as a source of low molecular weight CTs at level of 3.0 %DM of CTs equivalent, T3) supplemented with the same diet with T2 but added with polyethylene glycol (PEG, as tannin inactivator) as the control of T2, T4) supplemented with quebracho CT extract (UNITAN ATO, Buenos Aires, Argentina; 75-77 % tannins) in a concentrate as a source of high molecular weight CTs at level of 3.0 %DM of CTs equivalent, and T5) supplemented with the same diet with T4 but added with PEG as the control of T4.