

## Effects of Hydrolysable Tannin Supplementation on Nutrient Intake, Milk Production and Milk Somatic Cell Count in On-farm Dairy Cows

Tipwadee Prapaiwong<sup>1</sup>, Chalong Wachirapakorn<sup>1,\*</sup>, Chaiwat Jarassaeng<sup>2</sup>,  
Wuttikorn Srakaew<sup>3</sup> and Sukanya Poolthajit<sup>1</sup>

<sup>1</sup>Department of Animal Science, Faculty of Agriculture, Khon Kaen University,  
Khon Kaen 40002, Thailand

<sup>2</sup>Division of Theriogenology, Faculty of Veterinary Medicine, Khon Kaen University,  
Khon Kaen 40002, Thailand

<sup>3</sup>Department of Animal Science and Fisheries, Faculty of Science and Technology, Nan Campus,  
Rajamangala University of Technology Lanna, Nan 55000, Thailand

(\* Corresponding author's e-mail: chal\_wch@kku.ac.th)

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### Abstract

This study examined the effects of hydrolysable tannin (HT) supplementation on nutrient intake, milk production and milk somatic cell count in on-farm dairy cows. Fifty-four Holstein Friesian crossbred cows weighing 450 to 500 kg and were subjected to an experiment for 49 days. Dairy cows were divided into 3 groups, which were 1) the control group without HT supplementation and the HT supplementation groups, which received HT products (15 g/day), 2) one contains 3.15 g/day of HT and 3) another contains 6.30 g/day of HT. Hydrolysable tannin was extracted from sweet chestnut wood (*Castanea Sativa Mill.*). Dairy cows received HT products on the top of concentrate (22 % CP) in the morning after milking. Total mixed ration (TMR), pangola grass (*Digitaria eriantha*) hay and concentrate were offered according to animal nutrient requirements. The results showed that supplementation of HT has no influence ( $p > 0.05$ ) on the nutrient intake, milk yield and milk composition when compared to the control group. In addition, the increased levels of HT caused quadratic increases in fat:protein at pre-treatment and treatment periods ( $p < 0.05$ ;  $p = 0.06$ , respectively), while differential somatic cell count (DSCC) at treatment and post-treatment periods showed a quadratic decrease ( $p < 0.05$ ;  $p < 0.05$ , respectively), with supplementation of HT product containing 6.30 g HT/day ( $p < 0.05$ ) being the lowest. Therefore, it can be concluded that supplementation with HT product does not affect nutrient intake, milk yield, however, it could reduce DSCC and probably improve udder health in on-farm dairy cows.

**Keywords:** Hydrolysable tannin, Nutrient intake, Milk production, Milk somatic cell count, On-farm dairy cows

### Introduction

Using phytobiotics and medicinal plants as natural antimicrobial growth promoters in replacement to antibiotics in animal feeding has definitely many benefits for the progress of zootechnical efficiency parameters, suppression of specific diseases, antimicrobial and antioxidants activities, hypocholesterolemic effects, digestive enzymes enhancement and improvement of liver functions [1]. Researchers demonstrated that adding these plants to diet of poultry and other animals increased feed consumption, ratio of feed conversion and carcass yield [1]. In recent years, using natural products has increased, such as tannins, a heterogeneous group of water-soluble polyphenolic compounds of high molecular weight (500 - 3,000 Da unit) with as many as 20 hydroxyl groups that are present in plants, foods, and beverages [2]. Tannins exert several pharmacological effects, including antioxidant and free radical scavenging activity and antimicrobial, anti-cancer, anti-nutritional and cardio-protective properties, which provide beneficial effects on metabolic disorders and prevent the onset of several oxidative stress-related diseases [3]. Additionally, hydrolysable tannins (HT), as 1 group of tannins which are water extracted from sweet chestnut wood (*Castanea sativa Mill.*) have several effects as antioxidant, antimicrobial, and metal complexing agents [4].

Hydrolysable tannins have been used as feed additives [5] due to their biological properties as antibacterial agents. Hydrolysable tannins can inhibit extracellular microbial enzymes, deprive substrates, decrease essential mineral intake such as iron and zinc [6-8], and also reduce bacterial and enzymatic adhesion [9]. Supplementation of HT extract from sweet chestnut tree 0.49 % in diet had no effect on dry matter intake and milk yield in dairy cow [10], while Liu *et al.* [11] reported that HT extract supplemented 1 % of diet had no effect on intake, body weight, body conditional score, milk yield, milk composition and especially decreased somatic cell score (SCS). Ali *et al.* [12] also reported that supplementation of HT at 40 g/day improved in milk yield as well as reduction of somatic cell count (SCC) whereas there was no effect on milk composition. Moreover, Aguerre *et al.* [13] reported that adding 0.45 % tannin mixture (HT and condensed tannin (CT)) reduced feed efficiency but had a positive effect on milk protein content.

At present, dairy farmers mainly use antibiotics to prevent and treat bovine mastitis. Consequently, antibiotic residues can lead to microbial resistance to the disease. *Streptococcus agalactiae* showed rates of 19.4 % for penicillin resistance [14] and *Staphylococcus aureus* was also reported as having high rates of resistance to penicillin [14,15]. In Thailand, *Staphylococcus aureus*, is resistant to oxacillin and gentamicin, which are widely used in the treatment of bovine mastitis [16,17]. Information, however, is lacking regarding the treatment of subclinical bovine mastitis without antibiotics in Thailand. Our preliminary *in vitro* trial found that HT at concentrations of over 1,000 mg/mL can have a strong antibacterial effect against subclinical mastitis-causing bacteria and can be used for the control and protection of bovine mastitis [18]. Thus, our hypothesis was that HT supplementation can improve milk production and milk somatic cell count in the dairy farms. Therefore, the experiment objective was to study the effects of HT supplementation on nutrient intake, milk production and milk somatic cell counts in on-farm dairy cows.

## Materials and methods

### Animal ethics

The Animal Ethics Committee of Khon Kaen University based on the Ethic of Animal Experimentation of the National Research Council of Thailand approved the experimental procedure. Record no IACUC-KKU-86/2560.

### Animals, experimental design and feeding

Fifty-four Holstein Friesian crossbred cows with weighing of 450 - 500 kg and milk production of 15 - 20 kg/day of the experimental location at the Dairy Cattle Research and Development Center, Pak Chong District, Nakhon Ratchasima Province were randomly assigned to treatments in a completely randomized design. The experimental period was 49 days: For the first 7 days as pre-treatment period was not HT product supplemented from 8 to 42 days assigned as treatment period were HT product supplemented, and the post-treatment period from 43 to 49 days, no HT product supplementation was applied. There were 3 dietary treatments as the control group without HT supplementation, supplementation with HT product (commercial product A) at 15 g/day providing 3.15 pure HT g/day, and supplementation with HT product (commercial product B) at 15 g/day providing 6.30 pure HT g/day.

Hydrolysable tannin was derived from sweet chestnut wood (*Castanea Sativa* Mill.) provided by Animal Supplement and Pharmaceutical Co., Ltd.

Dairy cows received the HT products, on top of the concentrate (22 % CP) in the morning after milking. Total mixed ration (TMR), pangola grass (*Digitaria eriantha*) hay and concentrate were offered according to dairy cow nutrient requirements [19], while clean water and mineral blocks were available all day.

### Sample collection and analysis

Feed intake of TMR, pangola grass hay and concentrate were measured separately and recorded daily by weighing the offered and refused feed during the morning feeding to determine dry matter intake (DMI).

Feed samples were collected during days 36 to 40 of the experiment and pooled for analysis. Feed samples were dried at 60 °C for 48 h and ground through a 1-mm screen, followed by analysis for dry matter (DM), ether extract (EE), ash, and crude protein (CP) by the procedure based on [20], while fiber contents were determined using detergent analysis for neutral detergent fiber (NDF) and acid detergent fiber (ADF) by the method of [21].

Milk production of individual cow was recorded at each of the 2 daily milking (05.00 and 14.00 h) throughout the trial. Milk samples were collected in 2 portions once each week throughout the trial at the morning milking. The 1<sup>st</sup> portion of the milk sample at 100 mL was stored at 4 °C until milk composition analysis, which assessed fat, protein, lactose, total solids (TS), and solids-not-fat (SNF) contents, was conducted. Moreover, somatic cell count (SCC) using MilkcoScan FT 600 and Fossomatic 500 basic, respectively (Foss Electric, Integrated Milk Testing™) was performed, which transported to the Northeastern Veterinary Research and Development Center, Lower Zone (Surin) for milk composition analysis and identification of mastitis pathogens.

### Statistical analysis

All data were analyzed as a Completely Randomized Design (CRD). Data were analyzed using the model  $Y_{ij} = \mu + M_i + \epsilon_{ij}$ , where  $Y_{ij}$  = observation from animal  $j$ , receiving diet  $i$ ;  $\mu$ , the overall of mean,  $M_i$ , the mean effect of treatment ( $i = 1,2,3,4,5,6$ ),  $A_j$ , the effect of animal ( $j = 1,2,3,4,5$ ),  $\epsilon_{ij}$ , the residual effect which using the mixed linear models in proc Mixed [22]. Duncan's New Multiple Range Test [23] and orthogonal polynomial contrasts were determined the effects of hydrolysable tannins levels in the diets. All analyses were done with the SAS Institute Inc.® version 9.0 software. Difference was significant at  $p < 0.05$  and trends at  $p < 0.10$ .

## Results and discussion

### Chemical composition

In the present study, the chemical compositions of the diets are listed in **Table 1**. Results of the CP content in pangola grass hay was 2.57 %, which was lower than Wiyabot [24], who reported that the amount of protein in pangola grass hay was 4.46 % CP. Correspondingly, Tikam *et al.* [25] studied pangola grass hay from Thailand and found that the CP content varied from 3.1 - 10.5 % of DM. In addition, Chobtang *et al.* [26] reported that pangola grass hay contain CP, EE, NDF and ADF which are 7.0, 1.4, 69.5 and 36.6 %, respectively. Moreover, Kaewkunya *et al.* [27] indicated that the CP, EE, NDF and ADF in pangola grass hay were 4.3, 0.8, 69.5 and 35.1 %, respectively. Furthermore, these results of OM, EE, NDF and ADF are similar to the reports of Wiyabot [23], Chobtang *et al.* [26] and Kaewkunya *et al.* [27]. Variation in chemical compositions, particularly the CP concentration of pangola grass hay, was observed due to cutting age, the amount of applied nitrogen fertilizer or soil type and fertility [24].

TMR contained 11.18, 2.47, 50.19 and 24.93 % of CP, EE, NDF, and ADF, respectively. Concentrate used in this experiment contained 22.89, 4.59, 16.20 and 9.53 % of CP, EE, NDF and ADF contents, respectively. High CP content of concentrate which may be the result of the rare poor quality of the pangola grass hay provided. Furthermore, chemical composition of HT commercial products that provide different hydrolysable tannin concentrations is shown in **Table 1**.

**Table 1** The chemical composition of basal TMR, pangola hay and concentrate and hydrolysable tannin products.

Items	Pangola grass hay	Total mixed ration (TMR)	Concentrate	Hydrolysable tannin products (g)	
				Commercial product A	Commercial product B
Dry matter, %	95.49	47.40	93.62	95.62	93.82
<b>Chemical composition, % DM</b>					
Ash	5.81	6.51	9.57	34.16	17.18
OM	94.19	93.49	90.43	65.84	82.82
CP	2.57	11.18	22.89	1.47	1.32
EE	1.07	2.47	4.59	0.34	0.32
NDF	73.86	50.19	16.20	28.17	14.10
ADF	37.83	24.93	9.53	25.32	13.30

OM = Organic matter, CP = Crude Protein, EE = Ether Extract, NDF = Neutral detergent fiber, ADF = Acid detergent fiber

### Nutrient intake

The nutrient intake is presented in **Table 2**. There were no significant differences among treatments when compared with the control group for the nutrient intake of DM, OM, CP, EE, NDF, ADF, ME and

FE ( $p > 0.05$ ). The HT supplementation at 3.15 and 6.30 g/day was not significantly different ( $p > 0.05$ ) regarding all nutrient intake. This corresponds to Colombini *et al.* [28] and Lui *et al.* [29], who observed no significant effect of chestnut tannin extract (at 10 or 13 g/kg DM of the diet, respectively) on DMI of dairy cows. Similarly, Sliwinski *et al.* [30] reported no effect on feed intake and milk production when chestnut tannin was included at 0.49 % of dietary DM. According to Herremans *et al.* [31], no significant differences were detected between treatments in DMI, milk yield, and major milk components when HT from oak was included at 26 g/kg DM added in the forage of dairy cows, compared with the control group. Likewise, in McSweeney *et al.* [32], no effect on feed intake in sheep fed a diet of HT from *Terminalia oblongata* and *Clidemia hirta* was observed. These were given at 34 g/kg DM and more than 50 g/kg DM, respectively. Furthermore, Jeong *et al.* [33] reported that HT did not affect ( $p > 0.05$ ) body weight, average daily gain, DMI, and feed conversion ratio. When Hanwoo beef cows received commercial HT that was top-dressed to a concentrate mix at 3 g/kg based on the dry matter, medium or low hydrolysable tannins consumption levels appeared to be unaffected [34].

**Table 2** Effects of hydrolysable tannin supplementation on nutrient intake in the on-farm dairy cows.

Items	Hydrolysable tannin, g/day			SEM	Control vs HTs	Effect	
	0	3.15	6.30			L	Q
<b>DMI, kg/day</b>							
TMR	13.16	13.17	13.04				
Pangola hay	1.02	1.03	1.02				
Concentrate	1.26	1.10	1.30				
Total	15.44	15.29	15.32	0.14	0.43	0.54	0.60
<b>Nutrient intake, kg/day</b>							
OM	14.39	14.26	14.28	0.13	0.43	0.53	0.61
CP	1.78	1.74	1.76	0.03	0.52	0.72	0.48
EE	0.39	0.39	0.39	0.01	0.51	0.68	0.53
NDF	7.58	7.56	7.53	0.04	0.45	0.34	0.88
ADF	3.80	3.78	3.77	0.02	0.45	0.37	0.96
ME	39.96	39.47	39.64	0.46	0.46	0.61	0.55
FE	1.03	1.15	1.09	0.05	0.16	0.43	0.14

DMI = dry matter intake, TMR = total mixed ration, DM = dry matter, OM = organic matter, CP = crude protein, EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber, FE = Feed efficiency, L = Linear, Q = Quadratic

### Milk production and milk somatic cell count

The results obtained of milk production, milk composition and milk somatic cell count are shown in **Table 3** and **Figure 1**. The level of HT product supplementation had no effect ( $p > 0.05$ ) on milk yield, 4 % FCM, ECM and the milk composition, fat:protein, milk urea nitrogen, somatic cell count (SCC), somatic cell score (SCS), and differential somatic cell count (DSCC) when compared to the control group. Moreover, when increased, HT levels caused quadratic increases in fat:protein at pre-treatment and treatment periods ( $p < 0.05$ ;  $p = 0.06$ , respectively). Furthermore, DSCC at treatment and post-treatment periods show a quadratic decrease ( $p < 0.05$ ;  $p < 0.05$ , respectively), when increasing levels of HT (**Table 3**).

Several authors, Sliwinski *et al.* [30], Benchaar *et al.* [35] and Dschaak *et al.* [36], reported no changes in milk component concentration and yield when quebracho or chestnut tannins were added to the diet. However, Morales and Ungerfeld [37] reported that the duration of the tannins feeding period might also affect milk, which depends on the chemical type of tannins, the complexity of their interactions with dietary components, and the potential microbial adaptation to tannins.

Ali *et al.* [12] noted that supplementation of HT at 20 - 40 g/day combined with inclusion of Zn could reduce milk SCC. However, SCC and SCS in this experiment did not make a significant difference between treatments due probably to a level of HT may be less complementary. However, we observed that SCC and SCS parameters in cows received HT and after withdrawn HT could be stabilized while those parameters tended to increase in cows without HT supplementation (**Figures 1(a)** and **1(b)**).

In this trial, DSCC in cows supplemented with HT at 6.30 g/day ( $p < 0.05$ ) was the lowest (**Table 3** and **Figure 1(c)**). The DSCC indicates the percentage of polymorphonuclear neutrophil (PMN) combined with lymphocytes, and the proportions of macrophages can be calculated by  $100 - \text{DSCC}$  [38,39]. PMN, lymphocytes, and macrophages play an important role in the inflammatory responses within the mammary gland [40,39] found that low DSCC values in milk indicated low proportions of PMN and lymphocytes and thus high proportions of macrophages. Additionally, the DSCC parameter increased with the increase in mastitis signs, which is intuitive and evident [38]. In this way, diminished signs of mastitis at the treatment and post-treatment periods when the cows received HT at 6.30 g/day were due to the DSCC decreasing. This result of the DSCC and SCC, which is relevant with the DSCC values, occurred in a broad range from 34 to 79 % in samples with 400,000 cells/mL, and higher DSCC values (53 - 89 %) were found in samples with >400,000 cells/mL, as reported by [38].

**Table 3** The effects of hydrolysable tannin product supplementation on milk production and composition and somatic cell count in on-farm dairy cows.

Items	Hydrolysable tannin, g/day			SEM	Control vs HTs	Effect	
	0	3.15	6.30			L	Q
<b>Milk production, kg/day</b>							
Pre-treatment period	16.47	17.34	16.53	0.82	0.64	0.96	0.40
Treatment period	16.25	17.94	16.36	0.92	0.43	0.93	0.15
Post-treatment period	15.58	17.26	15.41	0.97	0.52	0.90	0.14
<b>4 % FCM, kg/day</b>							
Pre-treatment period	15.57	15.78	15.65	0.76	0.87	0.94	0.85
Treatment period	15.65	16.62	15.65	0.83	0.63	0.99	0.34
Post-treatment period	15.02	16.40	15.02	0.81	0.48	0.99	0.16
<b>Energy corrected milk (ECM), kg/day</b>							
Pre-treatment period	15.07	15.39	15.16	0.73	0.82	0.93	0.76
Treatment period	15.13	16.20	15.19	0.81	0.57	0.96	0.30
Post-treatment period	14.57	15.94	14.54	0.79	0.49	0.98	0.16
<b>Milk composition</b>							
<b>Fat, %</b>							
Pre-treatment period	3.62	3.43	3.67	0.11	0.62	0.74	0.12
Treatment period	3.74	3.61	3.77	0.12	0.76	0.84	0.34
Post-treatment period	3.79	3.77	3.92	0.18	0.81	0.62	0.70
<b>Protein, %</b>							
Pre-treatment period	2.96	2.99	2.93	0.06	0.99	0.74	0.56
Treatment period	2.99	3.05	2.98	0.06	0.80	0.89	0.46
Post-treatment period	3.04	3.11	3.07	0.08	0.61	0.78	0.59
<b>Lactose, %</b>							
Pre-treatment period	4.78	4.74	4.81	0.04	0.99	0.58	0.33
Treatment period	4.76	4.73	4.83	0.04	0.68	0.26	0.26
Post-treatment period	4.73	4.66	4.72	0.06	0.55	0.86	0.37
<b>Solid not Fat (SNF), %</b>							
Pre-treatment period	8.46	8.45	8.47	0.07	0.92	0.85	0.89
Treatment period	8.46	8.50	8.53	0.07	0.58	0.52	0.98
Post-treatment period	8.49	8.49	8.53	0.07	0.82	0.69	0.81
<b>Total solid (TS), %</b>							
Pre-treatment period	12.00	11.81	12.06	0.15	0.73	0.80	0.26
Treatment period	12.17	12.07	12.26	0.17	0.99	0.71	0.51

Items	Hydrolysable tannin, g/day			SEM	Control vs HTs	Effect	
	0	3.15	6.30			L	Q
Post-treatment period	12.23	12.22	12.39	0.24	0.80	0.64	0.76
<b>Fat:Protein</b>							
Pre-treatment period	1.22 <sup>ab</sup>	1.15 <sup>b</sup>	1.25 <sup>a</sup>	0.03	0.57	0.54	<0.05
Treatment period	1.25	1.19	1.27	0.03	0.46	0.79	0.06
Post-treatment period	1.25	1.21	1.26	0.03	0.73	0.78	0.25
<b>Milk urea nitrogen, mmol/L</b>							
Pre-treatment period	35.63	35.11	35.09	1.45	0.77	0.79	0.89
Treatment period	36.06	35.70	34.24	1.14	0.43	0.26	0.69
Post-treatment period	38.55	37.76	36.83	1.10	0.35	0.27	0.96
<b>SCC, ×10<sup>3</sup> cells/mL</b>							
Pre-treatment period	248.13	606.00	796.38	318.51	0.25	0.23	0.83
Treatment period	456.81	738.86	559.12	191.28	0.41	0.70	0.33
Post-treatment period	1390.38	799.94	826.08	547.94	0.39	0.47	0.64
<b>Somatic cell score (SCC)</b>							
Pre-treatment period	3.50	3.88	3.58	0.49	0.70	0.90	0.58
Treatment period	3.85	4.39	3.62	0.43	0.77	0.71	0.23
Post-treatment period	4.42	4.67	3.89	0.58	0.84	0.52	0.47
<b>DSCC, %</b>							
Pre-treatment period	51.73	61.73	54.25	6.76	0.45	0.79	0.29
Treatment period	52.53 <sup>b</sup>	69.56 <sup>a</sup>	54.19 <sup>ab</sup>	5.52	0.17	0.83	<0.05
Post-treatment period	57.81 <sup>ab</sup>	72.05 <sup>a</sup>	46.46 <sup>b</sup>	6.67	0.86	0.23	<0.05

ECM = milk×(0.38×% fat + 0.24×% protein + 0.17×% lactose)/3.17

Pre-treatment period = 1<sup>st</sup> week of experiment (no treatment)

Treatment period = average of 2<sup>th</sup> week - 6<sup>th</sup> week of experiment

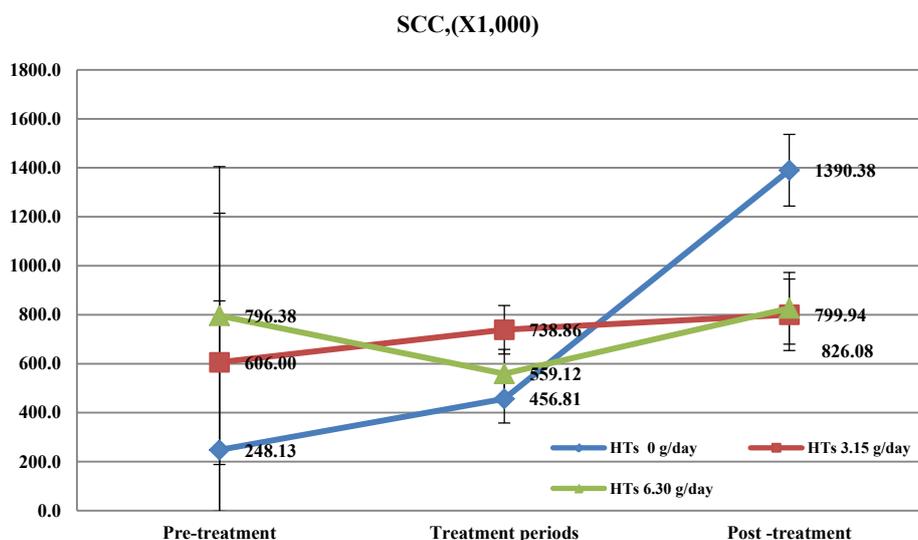
Post-treatment period = 7<sup>th</sup> week of experiment (no treatment)

Somatic cell count (SCC)

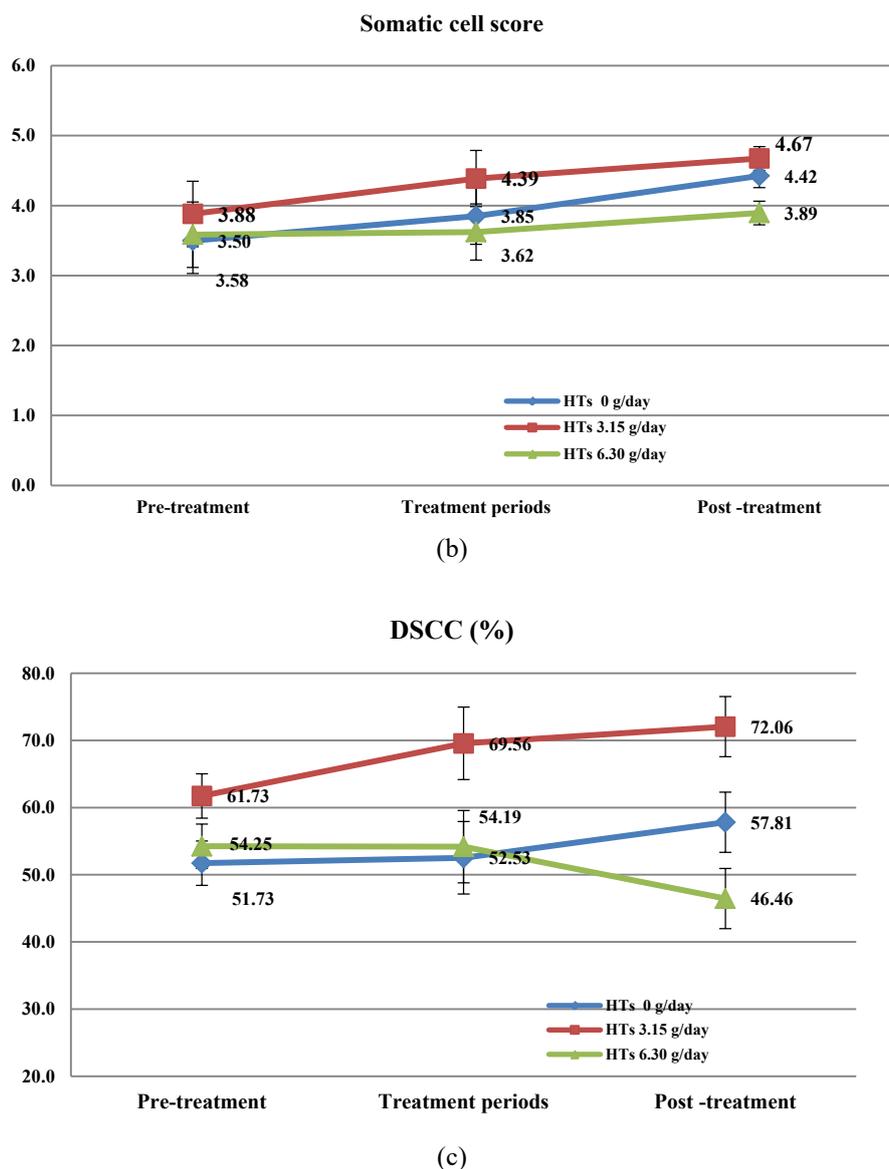
Somatic cell score (SCS) =  $\log_2(\text{SCC}/100,000) + 3$  [41]

Differential somatic cell count (DSCC)

L = Linear, Q = Quadratic



(a)



**Figure 1** Changes of somatic cell count (SCC) (a), somatic cell score (SCS) (b) and differential somatic cell count (DSCC) (c) in milk of cows fed different hydrolysable tannins levels.

## Conclusions

This experiment indicated that with regard to the level of HT supplementation at 3.15 - 6.30 g/day, it was found that there were no significant differences between the treatments of HT with respect to nutrient intake and milk production. However, on-farm dairy cows received HT at 6.30 g/day showed an improvement of DSCC values during treatment and post-treatment periods which were lower as compared to the others. This finding may help improve udder health in on-farm dairy cows possibly due to increase immune and then stabilize SCC by HT supplementation.

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## References

- [1] SH Masoudzadeh, M Mohammadabadi, A Khezri, RV Stavetska, VP Oleshko, OI Babenko, Z Yemets and OM Kalashnik. Effects of diets with different levels of fennel (*Foeniculum vulgare*) seed powder on DLK1 gene expression in brain, adipose tissue, femur muscle and rumen of Kermani lambs. *Small Rumin. Res.* 2020; **193**, e106276.
- [2] NZ de Jesus, H de Souza Falcão, IF Gomes, TJ de Almeida Leite, GR de Morais Lima and JM Barbosa-Filho. Tannins, peptic ulcers and related mechanisms. *Int. J. Mol. Sci.* 2012; **13**, 3203-28.
- [3] A Smeriglio, D Barreca, E Bellocco and D Trombetta. Proanthocyanidins and hydrolysable tannins: Occurrence, dietary intake and pharmacological effects. *Br. J. Pharmacol.* 2017; **174**, 1244-62.
- [4] S Miele, S Tegli, CG Izquierdo, M Cerboneschi and E Bargiacchi. Hydrolysable Tannins in Agriculture, Available at: <https://intechopen.com>, accessed July 2019.
- [5] HN Le Houerou. Browse in Africa. The current state of knowledge. *In: Proceedings of the International Symposium on Browse in Africa*, Addis Ababa, International Livestock Centre for Africa, Addis Ababa, Ethiopia. 1980.
- [6] R Puupponen-Pimia, L Nohynek, HL Alakomi and KM Oksman-Caldentey. Bioactive berry compounds- novel tools against human pathogens. *Appl. Microbiol. Biotechnol.* 2004; **67**, 8-18.
- [7] M Heinonen. Antioxidant activity and antimicrobial effect of berry phenolics a finnish perspective. *Mol. Nutr. Food Res.* 2007; **51**, 684-91.
- [8] P Buzzini, P Arapitsas, M Goretti, E Branda, B Turchetti, P Pinelli, F Ieri and A Romani. Antimicrobial and antiviral activity of hydrolysable tannins. *Mini-Rev. Med. Chem.* 2008; **8**, 1179-87.
- [9] BR Min, TN Barry, GT Attwood and WC McNabb. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: A review. *Anim. Feed Sci. Technol.* 2003; **106**, 3-19.
- [10] A Lavrenčič. Effect of hydrolyzable tannin extract on bovine milk production and composition. Sustainable grassland productivity. *In: Proceedings of the 21<sup>st</sup> ed. General Meeting of the European Grassland Federation*, Badajoz, Spain. 2006, p. 586-8.
- [11] H Liu, V Vaddella and D Zhou. Effects of chestnut tannin and coconut oil on growth performance, methane emission, ruminal fermentation, and microbial populations in sheep. *J. Dairy Sci.* 2011; **94**, 6069-77.
- [12] M Ali, HA Mehboob, MA Mirza1, H Raza1 and M Osredkar. Effect of hydrolysable tannin supplementation on production performance of dairy crossbred cows. *J. Anim. Plant Sci.* 2017; **27**, 1088-93.
- [13] MJ Aguerre, B Duval, JM Powell, PA Vadas and MA Wattiaux. Effects of feeding a quebracho-chestnut tannin extract on lactating cow performance and nitrogen utilization efficiency. *J. Dairy Sci.* 2020; **103**, 2264-71.
- [14] N Ramírez, J Cerón, M Jaramillo, O Arroyave and L Palacio. Diagnóstico de mastitis en el norte de Antioquia (pp. 69-78). *Memorias VII Seminario Internacional competitividad en carne y leche*. Medellín, 2010, p. 22.
- [15] JD Ruiz, NF Ramírez and O Arroyave. Determinación de concentraciones inhibitorias mínimas a algunos antibióticos de las bacterias aisladas de glándula mamaria bovina en San Pedro de los Milagros, Antioquia. *Rev. Colomb. Cienc. Pecu.* 2001; **14**, 76-87.
- [16] W Suriyasathaporn. Epidemiology of subclinical mastitis and their antibacterial susceptibility in smallholder dairy farms, Chiang Mai Province, Thailand. *J. Anim. Vet. Adv.* 2011; **10**, 316-21.
- [17] W Suriyasathaporn, V Chupia, T Sing-Lah, K Wongsawan, R Mektrirat and W Chaisri. Increases of antibiotic resistance in excessive use of antibiotics in smallholder dairy farms in northern Thailand. *Asian-Australas J. Anim. Sci.* 2012; **25**, 1322-8.
- [18] T Prapaiwong, W Srakaew, C Wachirapakorn and C Jarassaeng. Effects of hydrolyzable tannin extract obtained from sweet chestnut wood (*Castanea sativa* Mill.) against bacteria causing subclinical mastitis in Thai Friesian dairy cows. *Vet. World* 2021; **14**, 2427-33.
- [19] National Research Council. *Nutrient Requirement of Dairy Cattle*. 7th Revised Edition, 2001. The National Academies Press, Washington, DC.
- [20] AOAC. *Official method of analysis*. 16<sup>th</sup> Ed. Association of Official Agriculture Chemicals, Washington DC, USA, 1990.
- [21] HK Goering and PJ Van Soest. *Forage fiber analysis (apparatus, reagents, procedures and some applications)*, agricultural handbook no. 379. ARS USDA, Washington, DC, USA, 1970.
- [22] Statistical Analysis System Institute (SAS). *User's guide: Statistics version 9.0*. SAS Institute, Cary. 2002.

- [23] RG Steel and JH Torrie. *Principles and procedures of statistics: A biometrical approach*. 2<sup>nd</sup> ed. McGraw-Hill, New York, 1980, p. 633.
- [24] T Wiyabot, Pangola (*Digitaria eriantha*) hay as a roughage source in total mixed ration (TMR) on growth performance and meat quality of Thai native male goats in a tropical area. *Int. J. Agric. Technol.* 2018; **14**, 441-54.
- [25] K Tikam, C Phatsara, C Mikled, T Vearasilp, W Phunphiphat, J Chobtang, A Cherdthong and KH Südekum. Pangola grass as forage for ruminant animals: A review. *Springerplus* 2013; **2**, 604.
- [26] J Chobtang, W Angthong, N Khotprom, S Phojun and R Namsilee. Effect of quality of *Digitaria eriantha* hay on intake, digestibility and methane emission by beef cattle. *Khon Kaen Agr. J.* 2012; **40**, 166-9.
- [27] C Kaewkunya, W Meenongyai, D Vaduancai and W Puttaisong. The use of lablab bean as high-quality roughage on sheep productive performance. *Khon Kaen Agr. J.* 2013; **41**, 369-75.
- [28] S Colombini, G Colombari, GM Croveto, G Galassi and L Rapetti. Tannin treated lucerne silage in dairy cow feeding. *Italian J. Anim. Sci.* 2009; **8**, 289-91.
- [29] HW Liu, DW Zhou and K Li. Effects of chestnut tannins on performance and antioxidative status of transition dairy cows. *J. Dairy Sci.* 2013; **96**, 5901-7.
- [30] BJ Sliwinski, M Kreuzer, F Sutter, A Machmuller and HR Weststein. Performance, body nitrogen conversion and nitrogen emission from manure of dairy cows fed diets supplemented with different plant extracts. *J. Anim. Feed Sci.* 2004; **13**, 73-91.
- [31] S Herremans, V Decruyenaere, G Cantalapiedra-Hijar, Y Beckers and E Froidmont. Effects of hydrolysable tannin-treated grass silage on milk yield and composition, nitrogen partitioning and nitrogen isotopic discrimination in lactating dairy cows. *Animal* 2020; **14**, 771-9.
- [32] CS McSweeney, PM Kennedy and A John. Effect of ingestion of hydrolysable tannins in *Terminalia oblongata* on digestion in sheep fed *Stylosanthes hamata*. *Aust. J. Agr. Res.* 1988; **39**, 235-44.
- [33] S Jeong, M Lee, S Jeon, Y Kang, H Kang and S Seo. Effects of tannin supplementation on growth performance and methane emissions of Hanwoo beef cows. *Korean J. Agric. Sci.* 2018; **45**, 463- 73.
- [34] TN Barry and TR Manley. The role of condensed tannins in the nutritional value of lotus pedunculatus for sheep: 2. Quantitative digestion of carbohydrates and proteins. *Br. J. Nutr.* 1984; **51**, 493-504.
- [35] C Benchaar, TA McAllister and PY Chouinard. Digestion, ruminal fermentation, ciliate protozoal populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin, or *Yucca schidigera* saponin extract. *J. Dairy Sci.* 2008; **91**, 4765-77.
- [36] CM Dschaak, CM Williams, MS Holt, JS Eun, AJ Young and BR Min. Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows. *J. Dairy Sci.* 2011; **94**, 2508-19.
- [37] R Morales and EM Ungerfeld. Use of tannins to improve fatty acids profile of meat and milk quality in ruminants: A review. *Chil. J. Agric. Res.* 2015; **75**, 239-48.
- [38] M Damm, C Holm, M Blaabjerg, MN Bro and D Schwarz. Differential somatic cell count a novel method for routine mastitis screening in the frame of dairy herd improvement testing programs. *J. Dairy Sci.* 2017; **100**, 4926-40.
- [39] SK Wall, O Wellnitz, RM Bruckmaier and D Schwarz. Differential somatic cell count in milk before, during, and after lipopolysaccharide- and lipoteichoic-acid-induced mastitis in dairy cows. *J. Dairy Sci.* 2018; **101**, 5362-73.
- [40] LM Sordillo, K Shafer-Weaver and D De Rosa. Immunobiology of the mammary gland. *J. Dairy Sci.* 1997; **80**, 1851-65.
- [41] AK Ali and G Shook. An optimum transformation for somatic cell concentration in milk. *J. Dairy Sci.* 1980; **63**, 487-90.