

Ensiled Green Tea Waste as Partial Replacement for Soybean Meal and Alfalfa Hay in Lactating Cows

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ABSTRACT : The purpose of this study was to evaluate the effects of protein supplementation of green tea waste (GTW) on the performance of lactating cows. Another aim was to increase resource utilization and to eliminate any environmental negative impact from the tea waste. GTW from a beverage company was ensiled at a low pH (<4.0) and high acetic acid and lactic acid concentration, and it contained high crude protein (CP, 34.8%), total extractable tannins (TET, 9.2%) and condensed tannin (CT, 1.7%). Two experiments were conducted to investigate the palatability and performance in lactating cows fed GTW. In the palatability trial, three lactating cows were allocated to three dietary treatments in a 3×3 Latin square design. The animals were offered a total mixed ration (TMR) including GTW at rates of 0, 2.5 and 5.0% on a dry matter (DM) basis. Total DM intake was not different among the treatments. In the performance trial, four lactating cows were used in a 2×2 Latin square design with a 3 week sampling period. GTW was incorporated into TMR at a rate of 5.0% on a DM and 10.0% on a CP basis. Thus GTW replaced alfalfa hay and soybean meal at a level of 25.0% on a DM. DM and CP intake were not affected by the inclusion of GTW, whereas TET and CT intake were significantly increased ($p<0.001$). Milk production, milk composition and the efficiency of milk production were not altered by the GTW inclusion. Although ruminal pH and VFA, and blood urea nitrogen were not changed, ruminal $\text{NH}_3\text{-N}$ and plasma total cholesterol were relatively low in the GTW group, but not significantly different. The excretion of urinary purine derivatives and estimated MN supply were also not significantly affected by GTW treatment. It is therefore concluded that GTW can be used as a protein source without any detrimental effects on the performance of lactating cows. (*Asian-Aust. J. Anim. Sci.* 2004, Vol 17, No. 7: 960-966)

Key Words : Green Tea Waste, Lactating Cows, Milk Yield, Protein, Tannin

INTRODUCTION

Green tea is one of the major drinks in East Asia, some parts of the Middle East and North Africa (Graham, 1992). In Japan, consumption of green tea has been increasing remarkably in recent years. Beverage companies manufacturing various tea drinks produce about 100 thousand tons of tea-leaf waste annually, most of which is burned, dumped into landfills or used as compost. Tea leaves contain much nitrogen compounds, amino acids, tannins, polyphenols such as catechin, epicatechin gallate, epigallocatechin gallate, and vitamins (Yamamoto et al., 1997), suggesting that tea-leaf waste may have potential as an animal feed. A method should be developed to efficiently utilize tea waste as a feed resource with no negative environmental impact.

Green tea waste (GTW) may be considered as a valuable protein source consisting of 22-35% of crude protein (CP) (Yang et al., 2003; Kondo et al., 2004b). Whole-crop oat silage supplemented with GTW increased the CP content of the silage, and improved the nitrogen balance and ruminal $\text{NH}_3\text{-N}$ concentration in goats fed the silage (Kondo et al., 2004a). GTW could thus be a useful

protein source for animals on a feeding system employing low quality forage in protein-deficient areas. On the other hand, growing cows and lactating cows require high CP in their ration for an intensive livestock production system. In Japan, protein-rich commercial feeds such as soybean meal and alfalfa hay are often used in livestock production, but they are mostly imported. Byproducts that are produced from local agro- and food- industry could partially replace with commercial feedstuff (Chiou et al., 1998; Huang et al., 1999). And it would be both economically and environmentally beneficial to use such local byproducts instead of commercial feedstuff. While GTW contains high CP and may well meet these demands, it is known to contain a high proportion of tannins (Yang et al., 2003). Animals fed tannin-rich diets reportedly showed decreased their feed intake (Silanikove et al., 1994), increased fecal N excretion (Nunez-Hernandez et al., 1991), reduced digestibility and less ruminal degradability (Tolera et al., 1997; Woodward and Reed 1997). Thus, due attention must be given to tannin of GTW when it is used instead of commercial feed in livestock production. To date, only a few reports have evaluated the feed value of GTW.

The objective of this study was to evaluate the effects of partial replacement of soybean meal and alfalfa hay with GTW. GTW was offered to lactating cows as silage in this study because GTW itself is easily deteriorated after disposal by a beverage company. Two experiments were conducted in our investigation: the first aimed to test the

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Table 1. Chemical composition (% DM) of ensiled green tea waste (GTW), alfalfa hay and soybean meal

	GTW	Alfalfa hay	Soybean meal
Dry matter	19.6	87.4	87.4
Crude protein, CP	34.8	17.6	46.6
NDICP ¹	4.8	1.6	2.0
ADICP ¹	1.6	1.1	0.9
NDICP, % CP	13.8	9.2	4.3
ADICP, % CP	4.7	6.2	2.0
Ether extract	7.1	1.8	1.4
NDF	31.0	40.3	14.3
ADF	24.1	32.0	8.7
Total extractable phenolics	11.39	0.85	0.31
Total extractable tannins	9.23	0.16	<0.01
Condensed tannins	1.67	<0.01	<0.01
pH	3.97		
Lactic acid	2.0		
Acetic acid	7.0		
Propionic acid	1.8		
Butyric acid	0.4		
NH ₃ -N	0.04		

¹ NDICP: Neutral detergent insoluble crude protein, ADICP: acid detergent insoluble crude protein.

palatability of GTW substituted for a part of the total mixed ration (TMR) on the practical level. The second experiment evaluated feed intake, milk production, ruminal fermentation characteristics and the microbial nitrogen (MN) supply of lactating cows fed TMR in which soybean meal and alfalfa hay were replaced by GTW.

MATERIALS AND METHODS

Silage preparation

GTW was obtained from a local tea company, and packed into polyethylene bags and tying with string after removing air by a vacuum pump to close the upside of each bag. GTW silage for experiments was stored at ambient temperature and used more than one month after ensiling.

Palatability trial

Three lactating Holstein cows, with an initial body weight of about 584 kg, were used at Aichi Prefectural Agricultural College (Okazaki, Japan). The palatability trial was conducted in a 3×3 Latin square design in each phase of the experiment consisting of a 7 day adaptation period followed by a 3 day collection period. The experimental diet in control treatment was a total mixed ration (TMR), a 40:60 roughage to concentrate ratio, almost the same as the control diet in experiment 2. Forage included alfalfa hay and sudangrass hay. The concentrate was made from corn, wheat bran, barley, soybean meal, bean hull, cottonseed, brewer's grain, and dried beat pulp. In GTW treatments, the TMR was replaced with GTW silage at 0, 2.5 or 5.0% on a dry matter (DM) basis. The cows were milked twice a day

at 06:00 h and 15:00 h. The diets were offered *ad libitum* by giving a weighed amount twice a day at 07:00 h and 16:00 h, and orts were collected and weighed at 15:30 h every day.

Performance trial

Four lactating Holstein cows, with an initial body weight of about 590 kg were used at the same college as the palatability trial. Body weight was measured at the start and end of each period. The performance trial was conducted in a 2×2 Latin square design in each phase of the experiment consisting of a 2 week adaptation period followed by a 3 week collection period. Experimental diets were formulated iso-nitrogenous and iso-energenous TMR. GTW was incorporated into TMR at a rate of 5.0% on DM and 10.0% on a CP basis. Thus GTW replaced alfalfa hay and soybean meal at a level of 25.0% DM. The milking and feeding were done the same as for the palatability test. During collection periods, milk yield and feed intake were measured daily, while milk was sampled once a week for measurement of milk composition. At the end of the collection period, blood samples and rumen fluid were taken 5 h post-prandially. Blood samples were obtained from jugular vein by vacutainer tubes and centrifuged to get plasma. Rumen fluid was taken by a stomach tube, filtered through 4 layers cheesecloth and measured the pH. The spot urine samples were collected between 08:00 and 12:00 h on 5 consecutive days in the final week. The urine samples were given a pH below 3 with 10% H₂SO₄. Plasma, rumen fluid and urine samples were stored at -30°C until analysis.

Analytical methods

DM and organic matter (OM) were determined by drying the samples in an oven at 60°C for 48 h and by ashing at 550°C for 2 h, respectively. Feed and orts were freeze-dried and ground with a 1 mm screen, then used for the following analysis. Crude protein (CP) was analyzed by the Kjeldahl method, and the content of ether extract (EE) was determined by diethylether (AOAC, 1984). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Goering and Van Soest (1970). Neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP) were determined by the method of Licitra et al. (1996). Total extractable phenolics (TEPH), total extractable tannins (TET), and condensed tannins (CT) were analyzed by the methods of Makkar and Goodchild (1996). Twenty g of silage was macerated with 100 ml of distilled water. The macerate was filtered, and the filtrate was used to determine pH, lactic acid, volatile fatty acids (VFA), and NH₃-N. Lactic acid was measured colorimetrically (Barnett, 1951). For silage and rumen fluid, pH was measured potentiometrically. NH₃-N was determined by steam distillation (Ohshima et al., 1991), and volatile fatty acid (VFA) was detected with a gas

Table 2. Ingredients and chemical composition of total mixed ration including green tea waste (GTW) for lactating cows

	Control	GTW
Ingredients, % DM		
Sudangrass hay	18.5	18.5
Alfalfa hay	15.0	11.0
Soybean meal	4.6	3.6
Ensiled GTW	-	5.0
Corn	13.2	13.2
Barley	12.0	12.0
Beet pulp	10.2	10.2
Compound feed ¹	7.1	7.1
Soybean	4.8	4.8
Bean curd	4.5	4.5
Wheat bran	4.2	4.2
Cotton seed	2.3	2.3
Wet brewers' grain	2.2	2.2
Limestone	0.8	0.8
Salt	0.3	0.3
Magnesium oxide	0.2	0.2
Mineral mixture	0.1	0.1
Total	100.0	100.0
Calculated nutrient value		
Crude protein, % DM	17.0	17.0
Metabolic energy, MJ/kg	11.5	11.5
Analysed nutrient value, % DM		
Dry matter	53.2	52.2
Crude protein	16.6	16.9
NDICP ²	3.2	3.4
ADICP ²	1.1	1.1
NDICP, % of CP	20.4	20.1
ADICP, % of CP	6.9	6.9
Ether extract	4.1	4.3
NDF	39.1	39.3
ADF	21.5	21.3
Total extractable phenolics	0.56	1.09
Total extractable tannins	0.14	0.60
Condensed tannins	<0.01	0.15

¹ Compound feed: the mixture of corn, soybean meal, corn gluten meal etc.

² NDICP: Neutral detergent insoluble crude protein, ADICP: acid detergent insoluble crude protein.

chromatograph (GC-12A, Shimadzu Co., Japan.) using a FAL-M column (Shimadzu Co., Japan).

Microbial purine absorbed (MPA) by the animals was estimated from the daily excretion of purine derivatives (PD) based on the model described by Chen and Gomes (1992). The daily output of PD was estimated by the PD: creatinine ratio (mmol PD/mmol creatinine \times kg metabolic body weight) as described by Chen et al. (1995) and Abdulrazak et al. (1997). The supply of MN (i.e., entering to the small intestine) was calculated from MPA (Chen and Gomes, 1992). Urinary PD was analyzed as the sum of allantoin and uric acid excretions. The Allantoin concentration was determined by the method of Chen and Gomes (1992). Uric acid and creatinine were analyzed by a commercial kit (WAKO, Japan).

Milk composition, including milk fat, protein, solid non-fat and milk urea N (MUN) was measured using a Milk Scanner (FT6000, Foss Electric Co., Denmark). Blood urea N (BUN) and plasma total cholesterol were analyzed using a commercial kit (WAKO, Japan).

Statistical analysis

In the palatability trial, the data were analyzed by a one-way analysis of variance (ANOVA) and tested using Fisher's PLSD test, performed with a Statview for Windows (SAS Institute, 1992). In the performance trial, the data were expressed as the mean and standard error of the mean (SEM). Statistical comparisons were made with Student's *t*-test using a Statview for Windows (SAS Institute, 1992).

RESULTS AND DISCUSSION

Chemical composition of GTW

Table 1 shows the chemical composition of ensiled GTW, and alfalfa hay and soybean meal. GTW contained more CP (34.8% DM) than alfalfa hay (17.6% DM) but less than soybean meal (46.6% DM). Both NDICP and ADICP protein fractions in GTW were higher than in alfalfa hay and soybean meal. Licitra et al. (1996) defined that the fraction of NDICP degrades slowly, and that of ADICP is low biological availability. Higher NDICP and ADICP contents could be caused by heat treatment in the dry processing of tea leaves and/or the extraction of tea from the leaves with hot water. Licitra et al. (1996) mentioned that tannins would possibly increase the insoluble protein associated with the plant cell wall. Incorporating GTW in TMR as a substitute for alfalfa hay or soybean meal, therefore, would supposedly lower CP degradability. The EE contents were also higher in GTW than in any other feedstuff, possibly because diethyl ether extracted not only lipids but also pigments and some tannins (Cherney, 2000). Fiber fractions of NDF and ADF in GTW were higher than in soybean meal but lower than in alfalfa hay. GTW contained 11.4, 9.2 and 1.7% of TEPH, TET and CT, respectively. In terms of feedstuff characteristics, GTW is similar to tropical legume leaves with their high nitrogen and tannin contents (Abdulrazak et al., 2000a,b; Rubanza et al., 2003). Tea waste of low DM content possibly deteriorates easily after tea drink extraction at a beverage company. Ensiled GTW showed low pH (3.97) and high lactic acid (2.0% DM), indicating that GTW was well-preserved by ensiling. Table 2 shows the TMR ingredients in the control and GTW treatment and their chemical composition. When GTW was substituted for soybean meal and alfalfa hay at a level of 25.0% of DM, it corresponded to replacement of 5.0% of the DM and 10.0% of the CP overall. TEPH, TET and CT contents of TMR were higher in GTW treatment than control.

Table 3. Dry matter (DM) intake of lactating cows fed total mixed ration including green tea waste (GTW) at 0 (Control), 2.5 (GTW2.5) or 5.0 (GTW5.0) % of DM

	Control	GTW2.5	GTW5.0	SEM	Significance p =
DM intake (kg/d)	17.5	17.8	18.1	0.81	0.88
DM intake (g/kg W ^{0.75})	143.1	147.5	150.1	7.79	0.83

Table 4. Nutrient intake and performance of lactating cows fed total mixed ration including green tea waste (GTW)

	Control		GTW		Significance p =
	Mean	SEM	Mean	SEM	
Nutrient intake, kg/d					
Dry matter, DM	20.7	0.41	20.1	0.65	0.51
Crude protein, CP	3.4	0.06	3.4	0.12	0.83
Total extractable tannins, g/d	29.0	0.6	120.9	3.9	<0.001
Condensed tannins, g/d	2.2	0.02	28.9	2.27	<0.001
Performance, kg/d					
Milk yield	28.0	1.71	26.9	1.91	0.68
4% FCM yield	28.4	0.81	27.6	0.80	0.51
Milk protein yield	0.93	0.04	0.91	0.05	0.76
Milk Composition, %					
Fat	4.16	0.41	4.25	0.39	0.88
Protein	3.34	0.09	3.41	0.12	0.67
Solid non-fat	8.95	0.06	8.79	0.12	0.25
Efficiency, kg/kg					
DM intake/milk yield	0.81	0.07	0.83	0.09	0.87
DM intake/FCM yield	0.79	0.02	0.80	0.04	0.93
CP intake/FCM yield	0.77	0.06	0.81	0.09	0.75
CP intake/milk protein yield	23.1	1.21	23.6	2.17	0.85

Palatability trial

Table 3 shows the DM intake of dairy cows fed TMR including GTW at rates of 0, 2.5 and 5.0% of DM. The DM intake of cows fed TMR with GTW was slightly but not significantly increased with the increment of GTW. Ensiled GTW contained high amounts of lactic acid, acetic acid and CT. It is reported that the DM intake of silage was negatively correlated with the acetic acid and lactic acid concentrations (Jones et al., 1980). Furthermore, tannin decreased the feed intake in ruminants (Silanikove et al., 1994), probably due to less palatability or negative effects on digestion. In heifers, significant depression of feed intake was noted in the short term (within 5 days) after the ration was changed, where CT extracted from quebracho was given over 500 g/day (Landau et al., 2000). In sheep, high proanthocyanidin content (6.0-12.0% DM) in forage legume decreased voluntary feed intake, whereas a moderate level (2.0-4.0% DM) had no such effect (Aerts et al., 1999). It is supposed that the negative impact of tannin on feed intake was not critical for cows in the present study, since the GTW inclusion rates were low. Therefore, GTW at less than an inclusion rate of 5.0% DM in TMR was well accepted by cows during our experimental period.

Performance trial

Nutrient intake and performance of lactating cows fed

TMR including GTW at a rate of 5.0% on a DM basis are presented in Table 4. DM intake for 3 weeks was not significantly affected by inclusion of GTW as in the palatability trial. Thus, the CP intake was not altered between the treatments, but TET and CT intake was higher in GTW treatment than in the control (p<0.001). This was due to the higher tannin content of TMR including GTW. The cows fed GTW consumed only about 30 g CT/day, far less than the 500 g CT/day required to suppress feed intake in heifers (Landau et al., 2000). The performance of lactating cows (milk yield, 4% FCM yield and milk protein yield) was not significantly different between the two treatments. Furthermore, milk composition (fat, protein and solid non-fat) and efficiency (DM intake/milk yield, DM intake/FCM yield, CP intake/FCM yield and CP intake/milk protein yield) were also not affected by the inclusion of GTW in TMR. It is suggested that GTW is possibly substituted below the rate of 5.0% on a DM basis and 10.0% on a CP basis for commercial feeds in lactating cows without any negative effects on its performance.

Ruminal and blood characteristics, and milk urea nitrogen are given in Table 5. Ruminal pH was almost the same in either treatment. Ruminal NH₃-N was slightly but not significantly lower in cows fed GTW-treated TMR than control TRM. Each animal fed GTW-treated TMR showed lower ruminal NH₃-N levels than cows fed control TMR, possibly because the GTW slightly decreased ruminal

Table 5. Ruminal and blood characteristics, and milk urea nitrogen of dairy cows fed total mixed ration including green tea waste (GTW)

	Control		GTW		Significance p =
	Mean	SEM	Mean	SEM	
Ruminal characteristics					
pH	6.5	0.13	6.5	0.16	0.97
NH ₃ -N mg/dL	8.5	0.7	7.3	1.0	0.34
Total VFA mmol/L	103.8	8.3	109.2	10.7	0.70
VFA, molar %					
Acetate (A)	58.7	1.4	60.6	2.0	0.47
Propionate (P)	27.0	2.5	25.1	2.2	0.59
A:P	2.8	0.3	3.1	0.4	0.55
Butyrate	14.3	1.2	14.3	0.8	0.99
Blood characteristics mg/dL					
Blood urea nitrogen	16.1	1.0	16.5	1.2	0.83
Total cholesterol	276.1	26.5	244.1	18.5	0.40
Milk urea nitrogen mg/dL	13.8	0.3	13.2	1.3	0.66

Table 6. Concentrations of allantoin, urea and creatinine of spot urine samples, daily purine derivatives (PD) excretion and daily microbial N (MN) supply in lactating cows fed total mixed ration including green tea waste (GTW)

	Control		GTW		Significance p =
	Mean	SEM	Mean	SEM	
Concentration, mmol/L					
Allantoin	15.7	1.2	17.2	0.4	0.28
Urea	2.2	0.2	2.0	0.2	0.56
Creatinine	5.9	0.7	6.1	0.5	0.79
PD excretion, mmol/d	202.0	7.9	208.8	6.8	0.54
MN supply, g/d	133.3	7.6	139.0	6.8	0.60

degradation of protein. Previous reports showed that tannins at levels of 1.5-5.0% decreased protein degradation in rumen (Salawu et al., 1999; Santos et al., 2000). The lower NH₃-N concentration in the present experiment was probably due to the lower protein degradability in GTW than in alfalfa hay and soybean meal, and/or that free tannins from GTW reduced degradation of other protein in feed by making a complex with feed protein or enzyme-associated microorganisms. The VFA concentration and molar portion of VFA were not significantly different between treatments. BUN and MUN were also not affected by the inclusion of GTW. Hwang et al. (2000) reported that MUN should range between 11-17 mg/dl with a balance between protein and energy when the milk protein concentration is higher than 3.0%. In this light, the MUN level in our study was suitable with a protein and energy balance. The mean plasma total cholesterol concentration in the GTW group was lower than in the control group, but the difference was not statistically significant. However, from comparison of plasma cholesterol in each animal, the level was remarkably low in all the cows fed TMR included GTW. There are many reports that ingestion of green tea or its polyphenols decreased plasma total cholesterol by suppression of lipid absorption in the digestive tract of rats (Vinson and Dabbagh, 1998; Teddy et al., 1999; Raederstorff et al., 2003). Yang et al. (2003) reported that plasma total cholesterol decreased clearly in a chicken fed diet with GTW at rates of 1.0-2.0%. Therefore, it is suggested that GTW has the potential to decrease plasma

total cholesterol in ruminants, but the effect would be more moderate than for mono-gastric animals.

Concentrations of allantoin, urea and creatinine, PD excretion and MN supply are given in Table 6. PD excretion and MN supply were not altered by the inclusion of GTW. Microbial protein synthesis depends on synchronization of non-protein N and ATP supply to ruminal bacteria (Ørskov, 1982). Ben Salem et al. (1999) demonstrated that protein degradability and MN synthesis in ruminants were suppressed when the diet containing high tannin. In this study, VFA and NH₃-N from protein degradation were similar between treatments. It is supposed that the proportion of GTW in the whole diet was low and CT concentration of the whole diet was too low to work against MN synthesis. Another possibility is that tannins in GTW were different from the general types of tannins in tropical legumes (Ben Salem et al., 1999).

The estimated costs of the inclusion of GTW in TMR on milk production in this study are presented in Table 7. Daily income from milk was calculated based on the average of both treatments because parameters of milk price (yield, fat, solid non-fat) were not significantly different. The projected price of the GTW was put at -5 to 13 yen/kg on fresh weight. Table 7 indicates several different projected prices for the GTW, because it varies with the feed value of GTW and the demand. A disposal fee of 10 yen/kg is now charged. This is the amount the beverage company pays a waste disposal firm and the price is depending on the time and society. However, we assume that if a farmer receives the

Table 7. The economics (yen/day/head) of lactating cows fed total mixed ration including green tea waste (GTW)

	Control	GTW*				
		-5	0	5	10	13
Cost of alfalfa, soybean meal and GTW	270	200	200	230	260	280
Cost of total feed	1,140	1,060	1,060	1,090	1,120	1,140
Daily income from milk and GTW	2,320	2,350	2,320	2,320	2,320	2,320
Margin	1,180	1,290	1,260	1,230	1,200	1,180

* Assumption of the price of GTW, yen/kg fresh weight.

GTW directly, he will receive 5 yen/kg from the beverage company. Alfalfa hay and soybean meal are major protein sources of feed in dairy production, but they are costly and often imported. When the GTW price becomes 13 yen/kg, the profit would be almost the same as when using imported soybean meal and alfalfa hay at a milk price of 2,320 yen.

The results of the present study suggest that ensiled GTW could be used as a locally available feed resource in formulating TMR for lactating cows at 5.0% on a DM basis and could even replace alfalfa and soybean meal at a level of 25.0% on a DM basis without any detrimental effects on the performance of dairy cows. Replacing imported commercial feedstuffs with GTW could also save the energy for transporting imports and possibly reduce the environmental impact of burning the waste or using it for landfill.

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