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Effects of Green Tea (*Camellia sinensis*) Waste Silage and Polyethylene Glycol on Ruminal Fermentation and Blood Components in Cattle

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ABSTRACT : The effects of green tea (*Camellia sinensis*) waste silage and supplemental polyethylene glycol (PEG) on rumen fermentation and blood components were studied in cattle. Six Holstein steers were fed three diets in a 3×3 Latin square design, replicated twice. One diet was a control with no added silage, and the other two diets were supplemented (20% of the dry matter) with green tea waste silage either with (PEG) or without PEG (tea). Most of the fermentation parameters including major volatile fatty acids (VFA) were not affected by the diet treatments. The concentrations of high density lipoprotein cholesterol in the PEG group and urea nitrogen in the tea and PEG groups were greater than those in the control before morning feeding. The plasma 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid equivalent concentration was not different before morning feeding, but 3 h after morning feeding, its concentrations in both the tea and PEG groups were higher than in the control. Although the concentration of plasma vitamin A in the animals was not affected by feeding green tea waste silage, the concentrations of plasma vitamin E were significantly higher in the tea and PEG groups than in the control, both before and 3 h after morning feeding. The results from the present study suggest that feeding diets containing 20% of the dietary dry matter as green tea waste silage to Holstein steers has no negative impact on their ruminal fermentation, and increases their plasma antioxidative activity and concentration of vitamin E. (**Key Words** : Green Tea Waste, Ruminal Fermentation, Blood Components)

INTRODUCTION

Green tea (*Camellia sinensis*) is a popular beverage in Japan. Used green tea leaves from factories are disposed of as compost or incinerated by an industrial waste disposal contractor, which causes both an economical and environmental problem. The high water content (ca. 75%) of this waste limits its length of storage. Cai et al. (2001) have developed a method for preparing and conserving green tea leaf waste as silage by adding *Lactobacillus plantarum* and acremonium cellulase. Furthermore, they showed that a mixed silage preparation of green tea waste and corn was effective in improving fermentation quality (Cai et al., 2003). Kondo et al. (2004a, b, 2006) found that adding green tea waste enhanced the lactic acid fermentation of silage. The feeding value of green tea waste silage has been investigated with goats (Kondo et al., 2004c), sheep (Xu et al., 2003, 2004) and late-lactation Holstein cows (Eruden et al., 2003).

The effects of flavonoids, including the catechins found predominantly in tea, have been studied on a wide range of biological activities along with their effects on the promotion of health and prevention of disease in humans (Yamamoto et al., 1997; Dufresne and Farnworth, 2001; Nijveldt et al., 2001; McKay and Blumberg, 2002). These actions are almost certainly mediated in part by the radical-scavenging, antioxidant actions of tea polyphenols (Miyazawa, 2000). Green tea extracts improved microflora balance and showed antimicrobial effects against pathogenic bacteria in Holstein calves (Ishihara et al., 2001). Green tea waste still contains a lot of protein, tannin, caffeine, beta-carotene and vitamin E (Cai et al., 2001), and therefore, when fed to ruminants, might also help prevent disease.

The addition of 5 to 10% ensiled green tea waste in a

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total mixed ration (Kondo et al., 2004d) or timothy hay-based diets (Eruden et al., 2003) as a DM basis had no negative impact on the lactation performance in late-lactation dairy cows. However, crude protein digestibility in the tea fed group tended to be lower than in the control group (Eruden et al., 2003). The inverse relationship between a high tannin level in forage, and digestibility and nitrogen retention in ruminants, has been established (Silanikove et al., 1994, 1996a). Polyethylene glycol (PEG, MW 4000) is a polymer that binds tannins irreversibly, reducing the negative effects of tannins on food intake (Silanikove et al., 1994) and digestibility (Silanikove et al., 1996a). Protein may be released from the protein-tannin complex by an exchange reaction with PEG (Silanikove et al., 1996b). Therefore, the objectives of this study were to evaluate the effects of feeding green tea waste silage and supplemental PEG on nutrition and health in cattle by investigating ruminal fermentation and blood components.

MATERIALS AND METHODS

Animals and experimental design

Six Holstein steers were fed three diets in 21-d as a 3×3 Latin square design, replicated twice. One diet was a control with no added silage, and the other two diets were supplemented (20% of the DM) green tea waste silage with PEG (MW 4,000, Wako Pure Chemicals Industries, Ltd., Osaka, Japan) or without PEG (tea). The animals were cared for according to the Guide for the Care and Use of Experimental Animals (Animal Care Committee, National Institute of Livestock and Grassland Science), based on the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (1988).

Feeds and feeding

Fresh green tea waste (22% DM) was obtained from a beverage company and ensiled in 200 L drums with *Lactobacillus plantarum* (1.0×10^8/kg of fresh matter) and acremonium cellulase (0.02 g/kg of fresh matter). The drums were stored for 2 months in a room maintained at 20°C. The tea waste silage was mixed as the final ingredient in the concentrate portion of the diet. The diets were formulated to meet the maintenance requirements of the steers (Agriculture Forestry and Fisheries Research Council Secretariat, 1995). The steers were fed twice daily, at 09:30 and 16:30 h, in equal amounts. Water and mineral blocks (Koen-S; Nippon Zenyaku Kogyo, Co., Ltd., Fukushima, Japan) were available at all times.

Sampling and chemical analysis

Ruminal fluid was collected via the mouth using a rumen catheter (SANSHIN INDUSTRIAL Co., Ltd., Yokohama, Japan) just before and 3 h after the morning meal on the last day of the test period. The pH of the ruminal fluid was measured with a pH meter (D-24, HORIBA, Ltd., Tokyo, Japan). The ruminal fluid was separated from the feed particles through four layers of gauze, and centrifuged at 1,200×g for 15 min. A saturated perchlorate solution was added to the supernatant to deproteinize it, and the resultant fluid samples were then stored at -20°C until the assay.

Deproteinized ruminal fluid was neutralized with potassium hydroxide solution and centrifuged at 400×g for 10 min. The supernatant was subjected to ammonia (Weatherburn, 1967) and volatile fatty acid (VFA) analyses. Volatile fatty acid analysis was performed using a high performance liquid chromatography (HPLC) organic acid analysis system (Shimadzu, Kyoto, Japan). The supernatant was shaken with cation exchange resin (Amberlite, IR 120B H AG, ORGANO CORPORATION, Tokyo, Japan) and centrifuged at 6,500×g for 5 min. The supernatant was passed through a 0.45 μm filter under pressure, and the filtrate was then injected into an HPLC system. The analytical conditions were as follows: column, SCR-101H (7.9 mm×30 cm) attached to a guard column SCR(H) (4.0 mm×5 cm) (Shimadzu); oven temperature, 40°C; mobile phase, 4 mM p-toluenesulfonic acid aqueous solution; reaction phase, 16 mM Bis-Tris aqueous solution containing 4 mM p-toluenesulfonic acid and 100 μM ethylenediaminetetra-acetic acid; flow rate of the mobile and reaction phase, 0.8 ml/min; detector, conductivity detector (CDD-6A, Shimadzu).

Blood was taken from the jugular vein into a heparinized tube just before and 3 h after the morning meal on the last day of the test period. The samples were centrifuged at 1,200×g for 15 min. The plasma was separated and stored in a plastic tube and frozen at -80°C until analysis was performed. Plasma concentrations of glucose, urea nitrogen, triglyceride, total-, high density lipoprotein (HDL)-, low density lipoprotein (LDL)-cholesterol, total protein and non-esterified fatty acid (NEFA) were determined in an autoanalyser (Hitachi 7070, Hitachi, Ltd., Tokyo, Japan) using commercial kits supplied by Wako Pure Chemicals Industries, Ltd. (Osaka, Japan) for all metabolites. Plasma insulin and glucagon concentrations were measured using radioimmunoassay commercial kits (^{125}I-insulin Eiken, Eiken Chemical Co., Ltd., Tokyo, Japan and ^{125}I-glucagon kit Daiichi, TFB, Inc., Tokyo, Japan, respectively).

Plasma antioxidative activity was measured by commercial kits (Total Antioxidant Status, Randox Laboratories Ltd., Antrim, UK) which used 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, registered trademark of Hoffman-La Roche) equivalent

Table 1. Ingredients and chemical composition of experimental diets

Item	Treatment			Green tea waste
	Control	tea	PEG	
Ingredients (% of DM)				
Timothy hay	49.6	39.7	38.5	
Corn	40.0	32.0	31.0	
Soybean meal	9.9	7.9	7.7	
Green tea waste silage	0.0	19.8	19.2	
Mineral supplements	0.5	0.6	0.6	
PEG	0.0	0.0	3.0	
Chemical composition, % of DM				
Organic matter	94.9	95.0	95.2	96.8
Crude protein	13.1	17.1	17.1	33.2
Crude fiber	20.4	20.2	19.6	19.5
Ether extract	2.8	3.7	3.7	7.3
Acid detergent fiber	21.3	21.3	20.8	22.4
Neutral detergent fiber	37.3	37.3	35.9	29.9
Crude ash	4.6	4.4	4.3	3.2
Energy (MJ/kg DM)	18.8	19.7	19.4	23.5

Source: from Eruden et al., 2004.
PEG: 4,000 molecular weight polyethylene glycol.
DM = Dry matter.

concentrations as a parameter.

Plasma vitamin A and E analyses were performed using HPLC (JASCO Corp., Tokyo, Japan). The sample preparations were all carried out in light-resistant glass tubes to minimize the light-induced degradation of vitamins. A 0.5 ml volume of distilled water and 1.0 ml of 100% ethanol were added to 0.5 ml of plasma. The vitamins were then extracted with 5.0 ml of hexane by agitating on a mixer for 10 min. The tubes were centrifuged at 700×g for 10 min, and 4.0 ml of the hexane layer were transferred to another glass tube and evaporated to dryness using a centrifugal evaporator (Model CVE-2000, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The residue was dissolved in 100 μl of isopropyl alcohol by mixing for 30 s, and a 20 μl aliquot was injected into the chromatograph.

The analytical conditions were as follows: pump, Model PU-980 (JASCO); injector, Model 851-AS (JASCO); column, Shim-pack CLC-ODS (6.0 mm×15 cm) attached to a guard column Shim-pack G-ODS (10 mm×4 cm; Shimadzu); oven temperature, vitamin E at 40°C and vitamin A at 45°C; mobile phase, vitamin E in methanol and vitamin A in methanol and sodium acetate (9:1 v/v); flow rate of the mobile phase, 1.0 ml/min; detector, fluorescence detector (Model FP-920, JASCO); excitation wavelength, vitamin E at 296 nm and vitamin A at 340 nm; emission wavelength, vitamin E at 325 nm and vitamin A at 460 nm.

Statistical analyses

All data were analyzed by the general linear model procedure (SAS, 1988) for a replicated (n = 2) 3×3 Latin square design. The model was as follows: $y_{ijk} = m+T_i+P_j+C_k+TPC_{ijk}+e_{ijk}$ where y_{ijk} = the dependent variable for the cow on treatment i during period j; m = the overall mean; T_i = the treatment effect (i = 1 to 3); P_j = the period effect (j = 1 to 3); C_k = the cow effect (k = 1 to 5); TPC_{ijk} = the interaction of treatment×period×cow and e_{ijk} = residual error. Within each sampling time (before and 3 h after morning feeding), the statistical significance of difference between pairs of treatments was assessed by the Tukey's test following a significant main effect. Data are presented as the least squares means, and were considered statistically different if $p<0.05$.

RESULTS AND DISCUSSION

During the adaptation period to the experimental diets, one steer became inappetent and we therefore eliminated it from this study. Furthermore, at 3 d before sampling, another steer which was being fed the control diet escaped from its stanchion and ate some of the excess diet, and thus we omitted its data from the control data. Thus the number of cattle used in this study was 4 in the control group and 5 in both the tea and PEG treatment groups.

The ingredients and chemical composition of the experimental diets (Table 1) and fermentation quality of the green tea waste silage (Table 2) were the same as shown in our previous report (Eruden et al., 2004). The pH, ammonia, total VFA concentration, molar ratios of acetate, propionate, butyrate, and acetate to propionate in the rumen fluid were not affected by the diet treatments, though there were differences in the molar ratios of isobutyrate ($p<0.05$), isovalerate ($p<0.01$), and valerate ($p<0.01$) to the total VFA concentration at 3 h after morning feeding (Table 3). Green tea's antibacterial properties against a variety of gram-positive and gram-negative species have been demonstrated (Chou et al., 1999). Therefore, our supposition was that the antimicrobial activity of green tea might have negative effects on protozoa and bacteria and inhibit the production of VFA in rumen. However, other than differences among experimental diets in isobutyrate, isovalerate and valerate proportions at 3 h after morning feeding, the treatments had no effect on individual VFA proportions, pH, or the

Table 2. Fermentation quality of green tea waste silage [1]

pH	DM (%)	Organic acid composition (% FM)				VBN [2] (%DM)
		Lactic acid	Acetic acid	Propionic acid	Butyric acid	
3.6	22.1	1.4	0.9	0	0	0.05

Source: from Eruden et al. (2004).
[1] Silage stored for 360 d. [2] VBN: Volatile basic nitrogen.

Table 3. Effect of feeding green tea waste silage on ruminal fermentation in Holstein steers before and 3 hours after morning feeding

	Treatment						p-value
	Control		Tea		PEG		
	Lsmeans	SEM	Lsmeans	SEM	Lsmeans	SEM	
n	4		5		5		
Before morning feeding							
pH	6.9	0.2	7.0	0.1	7.0	0.1	0.6800
ammonia-N (mg/dl)	15.6	1.2	17.8	1.0	18.8	1.1	0.1854
Total VFA (mM)	90.5	8.2	85.8	6.9	83.3	7.0	0.7826
VFA (mol/100 mol)							
Acetate	65.5	0.9	63.8	0.7	64.0	0.8	0.3435
Propionate	17.4	0.6	18.4	0.5	17.6	0.5	0.4615
Isobutyrate	1.4	0.1	1.5	0.1	1.7	0.1	0.1901
Butyrate	12.1	0.6	12.6	0.5	12.9	0.5	0.6070
Isovalerate	2.4	0.2	2.6	0.1	2.7	0.1	0.3034
Valerate	1.2	0.0	1.1	0.0	1.1	0.0	0.9939
Acetate:propionate	3.8	0.2	3.5	0.1	3.7	0.1	0.4191
3 h after morning feeding							
pH	6.5	0.2	6.8	0.2	6.6	0.2	0.5512
ammonia-N (mg/dl)	13.9	1.7	13.6	1.4	13.6	1.4	0.9857
Total VFA (mM)	101.0	7.8	86.8	6.6	87.7	6.7	0.3544
VFA (mol/100 mol)							
Acetate	65.2	1.1	66.7	1.0	66.4	1.0	0.5904
Propionate	18.4	0.7	17.6	0.6	17.8	0.6	0.6952
Isobutyrate	1.1^{a}	0.0	1.0^{b}	0.0	1.0^{ab}	0.0	0.0390
Butyrate	12.2	0.5	12.2	0.4	12.3	0.4	0.9802
Isovalerate	1.9^{A}	0.1	1.5^{B}	0.1	1.5^{B}	0.1	0.0044
Valerate	1.2^{A}	0.0	1.0^{B}	0.0	1.0^{B}	0.0	0.0048
Acetate:propionate	3.6	0.2	3.8	0.2	3.7	0.2	0.6466

SEM = Standard error of mean. ^{a,b} $p<0.05$; A, B: $p<0.01$.

concentration of NH_3-N. Although we did not count the protozoal and bacterial numbers in this study, it would appear that feeding green tea waste silage at 20% of DM in diets had no negative impact on ruminal fermentation in Holstein steers.

The plasma urea N on the tea and PEG treatments was significantly higher ($p<0.05$) than that on the control diet both before and after feeding (Table 4). The CP content of both the tea and PEG diets was greater than the control diet, as the green tea waste contained a lot of CP (Table 1). Urea N in the plasma reflects the dietary CP content, as excess ruminal ammonia enters the blood and is converted to urea in the liver, whereas the concentration of NH_3-N in the rumen was not different between the diets in this study. Nitrogen excretion in the urine on the tea and PEG diets was significantly greater ($p<0.05$) than that on the control diet (Eruden et al., 2004). Kondo et al. (2004b) reported that the green tea waste silage increased *in vitro* ruminal gas production, and that this was probably due to better fermentability of the green tea waste itself. This good fermentability of the green tea waste may promote nitrogen absorption without increasing NH_3-N in the rumen. Adding PEG to the green tea waste, which contained a large amount of tannin, had no effect on nitrogen availability to Holstein steers in this study.

The plasma concentrations of NEFA increased significantly in the tea and PEG treatments ($p<0.05$) at 3 h after morning feeding, and tended to be higher ($p = 0.0842$) in the tea and PEG groups before morning feeding (Table 4). There were no significant differences in net energy balance of the control, tea, and PEG diets (Eruden et al., 2004). All NEFA values were low compared with periparturient cows (Holcomb et al., 2001) and no significant differences were observed in plasma concentrations of glucose, liver enzymes, insulin, and glucagon between the treatments. Furthermore, judging from the results of the energy balance trial, the steers might not be in a serious energy deficient status when consuming the tea and PEG diets.

The main functions of HDL cholesterol are to deliver cholesterol to tissues for primary steroidogenesis (liver, ovary, testis and adrenal gland) or membrane synthesis (a wide variety of tissues such as fibroblasts), and to take up free cholesterol from extrahepatic tissues and transport it to the liver or steroidogenic tissues; thereafter, HDL cholesteryl esters are transferred to LDL (Bauchart, 1993). Low levels of HDL cholesterol were associated with increased mortality from cardiovascular and coronary heart disease in humans (Wilson et al., 1988). The concentration of HDL cholesterol on the tea treatment was significantly greater ($p<0.05$) than that in the control only at 3 h after the

Table 4. Effect of feeding green tea waste silage on plasma metabolites and hormones in Holstein steers before and 3 hours after morning feeding

Item	Treatment						P-value
	Control		Tea		Peg		
	Lsmeans	SEM	Lsmeans	SEM	Lsmeans	SEM	
n	4		5		5		
Before morning feeding							
NEFA (μEq/L)	106.7	17.6	162.6	14.9	159.6	15.1	0.0842
GOT (IU/L)	57.6	5.9	63.5	5.0	68.0	5.0	0.4145
GPT (IU/L)	19.2	0.8	18.5	0.7	18.5	0.7	0.7641
Triglycerides (mg/dl)	27.5	2.5	27.9	2.1	30.7	2.2	0.5397
Total cholesterol (mg/dl)	72.5	4.0	77.3	3.4	83.0	3.4	0.1756
HDL cholesterol (mg/dl)	47.5^{a}	2.1	51.4^{ab}	1.8	55.9^{b}	1.8	0.0490
LDL cholesterol (mg/dl)	16.3	2.3	17.1	1.9	17.5	2.0	0.9099
LDL/HDL ratio	0.34	0.03	0.33	0.03	0.31	0.03	0.7465
Urea nitrogen (mg/dl)	10.8^{a}	0.8	15.0^{b}	0.7	14.4^{b}	0.7	0.0128
Glucose (mg/dl)	77.3	2.3	73.9	2.0	74.7	2.0	0.5430
Total protein (g/dl)	7.9	0.1	8.1	0.1	8.0	0.1	0.2497
Glucagon (pg/ml)	107.3	18.1	93.7	16.2	93.9	15.7	0.8135
Insulin (μIU/ml)	9.9	2.4	8.8	2.1	12.3	2.1	0.4912
I/G	2.2	0.4	2.2	0.3	3.1	0.3	0.1769
3 h after morning feeding							
NEFA (μEq/L)	70.1^{a}	10.1	110.7^{b}	8.6	98.7^{b}	8.6	0.0497
GOT (IU/L)	58.7	6.1	64.4	5.2	69.1	5.2	0.4371
GPT (IU/L)	18.9	1.1	18.9	0.9	18.9	0.9	0.9998
Triglycerides (mg/dl)	27.2	3.7	29.6	3.1	28.8	3.1	0.8817
Total cholesterol (mg/dl)	71.1	3.6	78.6	3.0	83.6	3.0	0.0784
HDL cholesterol (mg/dl)	46.5^{aA}	1.9	51.2^{b}	1.6	55.9^{B}	1.6	0.0212
LDL cholesterol (mg/dl)	16.9	2.3	17.4	2.0	17.7	2.0	0.9550
LDL/HDL ratio	0.36	0.03	0.34	0.03	0.31	0.03	0.5353
Urea nitrogen (mg/dl)	12.3^{a}	0.8	15.9^{b}	0.7	15.1^{b}	0.7	0.0337
Glucose (mg/dl)	80.3	3.0	74.3	2.5	73.0	2.5	0.1982
Total protein (g/dl)	7.7	0.1	8.2	0.1	8.0	0.1	0.0544
Glucagon (pg/ml)	111.7	17.1	107.4	14.5	95.5	14.6	0.7266
Insulin (μIU/ml)	10.6	1.8	9.9	1.5	9.0	1.5	0.7589
I/G	2.3	0.3	2.2	0.3	2.4	0.3	0.7875

NEFA: non-esterified fatty acid, GOT: Glutamic oxaloacetic transaminase, GPT: Glutamic pyruvic transaminase, HDL: high density lipoprotein, LDL: low density lipoprotein.
I/G: molar ratio of insulin to glucagons.
SEM = Standard error of mean. ^{a,b} $p<0.05$: A, B: $p<0.01$.

morning feeding (Table 4). Correspondingly, in the PEG group the concentration of HDL cholesterol was significantly greater both before ($p<0.05$) and 3 h after ($p<0.01$) the morning feeding than in the control. However, the plasma concentrations of total and LDL cholesterol, GOT, GPT and the ratio of LDL to HDL cholesterol were not significantly affected by feeding green tea waste silage to Holstein steers in this study. Serum total cholesterol levels in humans have been found to be inversely related to the consumption of green tea, while no association was noted with serum triglycerides and HDL cholesterol using cross-sectional data on males (Kono et al., 1992) and healthy Japanese male and female workers (Tokunaga et al., 2002). Epidemiological studies suggest that drinking multiple cups of green tea per day lowers LDL cholesterol in mild to moderate hypercholesterolemic adults (Maron et al., 2003). We cannot conclude whether or not higher HDL cholesterol in the plasma of cattle fed green tea waste silage is beneficial for the health of cattle, because their lifetime is too short for chronic cardiovascular disease and arteriosclerosis to be of concern. Most metabolic diseases occur during the periparturient period, and may be caused by fatty liver in cattle; consequently, more studies are needed to determine the effect feeding green tea waste silage has on the blood concentrations of apoB-100, apoA-I, and apoC-III and the activity of lecithin: cholesterol acyltransferase, which are useful markers for early diagnoses of fatty liver and related diseases (Katoh, 2002).

The Trolox (a registered trademark of Hoffman-La Roche) equivalent antioxidant capacity (TEAC) assay of Miller et al. (Miller et al., 1993b; Rice-Evans and Miller, 1994) has been developed. The TEAC assay is based on the

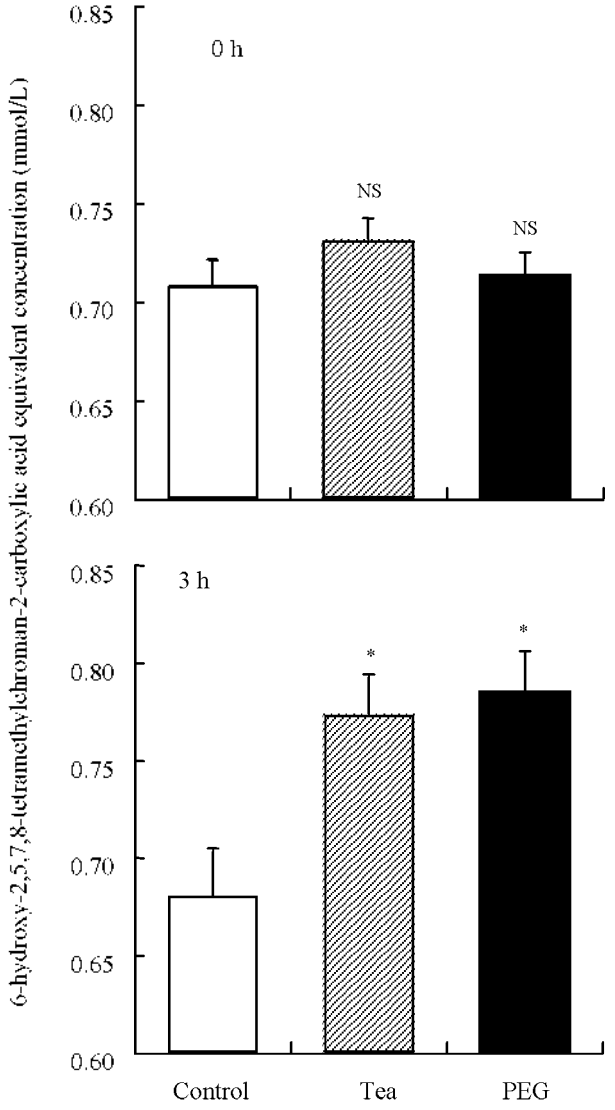

Figure 1. Effect of feeding green tea waste silage on plasma antioxidative activity in Holstein steers before (0 h) and 3 hours after (3 h) morning feeding (NS: No significant difference compared to the value in control, * $p<0.05$ compared to the value in control). Means±S.E.M.= standard error of mean.

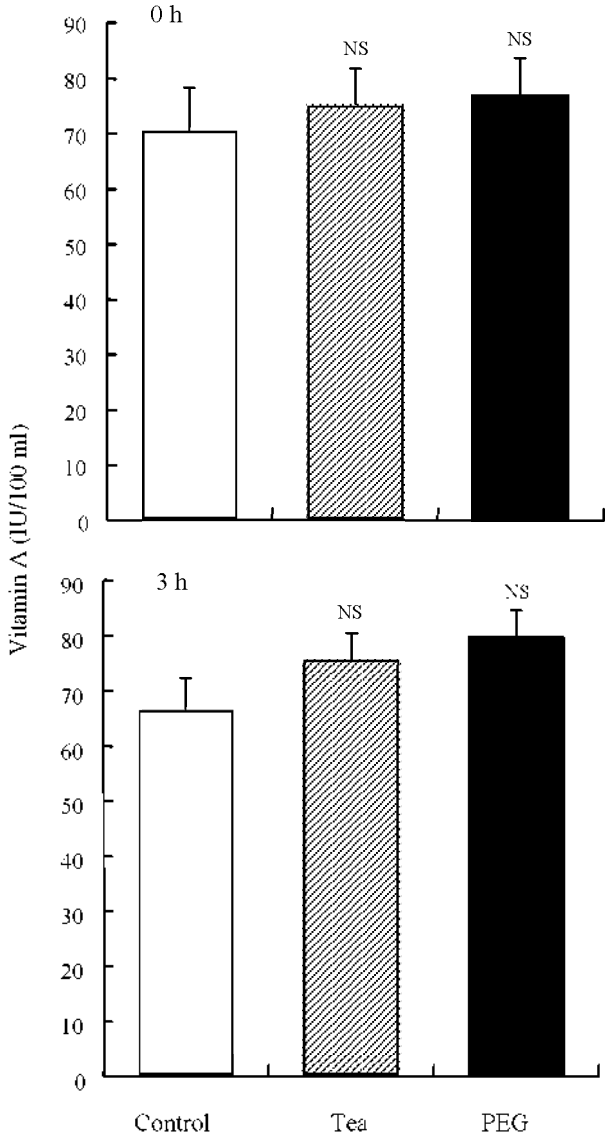

Figure 2. Effect of feeding green tea waste silage on plasma vitamin A concentration in Holstein steers before (0 h) and 3 hours after (3 h) morning feeding (NS: No significant difference compared to the value in control). Means±S.E.M. = standard error of mean.

inhibition by antioxidants of the absorbance of the radical cation of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate; ABTS), and has been commercialized by Randox Laboratories. This method has produced useful information regarding antioxidant activities *in vivo* in human studies. Sung et al. (2000) found a significant increase in the total antioxidant capacity of human plasma after the ingestion of green tea, as measured with the TEAC assay. The Trolox equivalent concentration was not significantly different before morning feeding when green tea waste silage was added, but the Trolox equivalent concentrations in both the tea and PEG groups were significantly higher ($p<0.05$) than that in the control at 3 h after morning feeding (Figure 1). The concentration of plasma vitamin A was not affected by feeding green tea waste silage (Figure 2). The concentration of plasma vitamin E was significantly increased in the tea ($p<0.05$) and PEG ($p<0.01$) groups before and ($p<0.01$) at 3 h after morning feeding (Figure 3). From these results, it is most likely that vitamins A and E did not affect the plasma antioxidant status in this study, because no differences in their plasma concentrations were observed before or after feeding when green tea silage was used to supplement diets for Holstein steers. The Trolox equivalent concentrations in the plasma of steers given green tea waste silage in their diets was significantly higher than when they were fed a control diet at only 3 h after feeding. Although feeding green tea waste silage as 10% of a diet on a DM

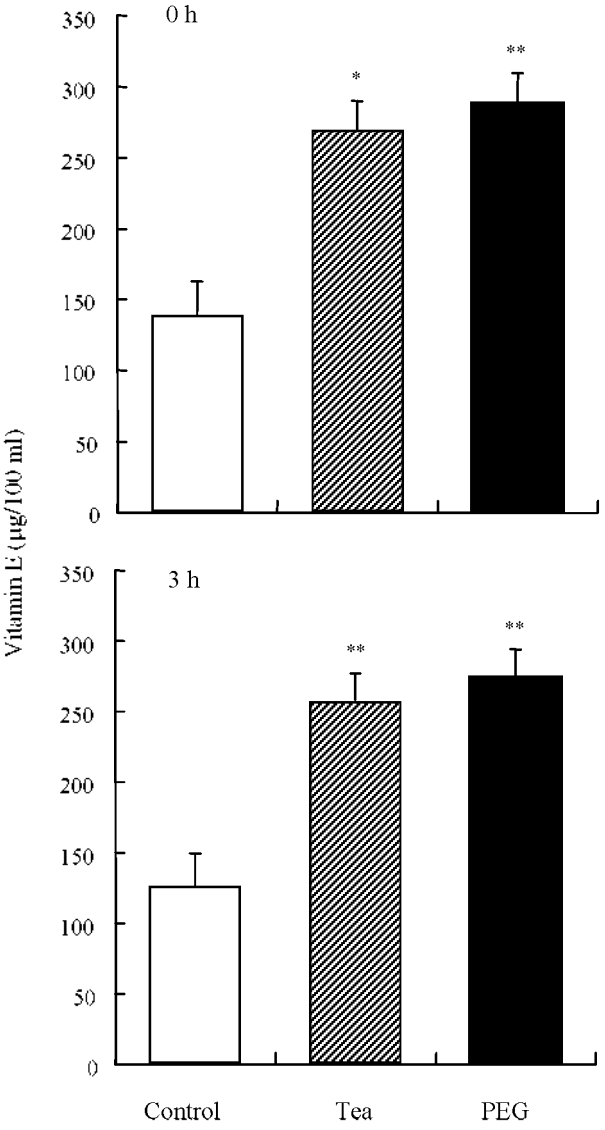

Figure 3. Effect of feeding green tea waste silage on plasma vitamin E concentration in Holstein steers before (0 h) and 3 hours after (3 h) morning feeding (* $p<0.05$, ** $p<0.01$ compared to the value in control). Means±S.E.M. = standard error of mean.

basis with late-lactation cows had no effect on the plasma antioxidative activity by TEAC assay before feeding in our previous studies, we observed that the plasma antioxidative activities were significantly higher than those of the control at 3 and 6 h after morning feeding, with no differences in the plasma concentration of vitamins A and E (Eruden et al., 2003, data not shown). It would appear that the major antioxidant in green tea waste other than vitamins A and E is catechine. The consumption of green tea causes a rapid increase in the plasma antioxidant power in humans (Benzie et al., 1999). Furthermore, in a human study, the plasma EGCg reached a maximum at 1-3 h after oral intake, and then gradually decreased (Nakagawa et al., 1999). Given that there was a significant increase in the Trolox equivalent concentration in the steers' plasma at 3 h after the green tea waste silage feeding in this study, the catechins could be the possible agent.

In humans, the plasma catechin levels increased rapidly during repeated tea consumption, but decreased significantly during sleep, when no tea was being consumed (Van het Hof et al., 1999). When the dosage was increased from 1.5 to 3.0 g, the maximum plasma concentration of catechins increased from 2.7 to 3.4 fold, but increasing the dose to 4.5 g did not significantly increase the maximum plasma concentration of catechins, which suggested a saturation phenomenon (Yang et al., 1998). Although greater plasma antioxidative activity was observed 3 h after the feeding of green tea waste silage in this study, there may be some differences in the bioavailability of catechins and other nutrients from green tea leaves between ruminants and humans, and rats or mice, due to the different types of supplements ingested. In this study, the green tea leaves were brewed in hot water (green tea waste), and in studies on human health, green tea extract or beverages were used.

In this study, each Holstein steer received 53.5 mg/kg of total catechins calculated from the following data: 20% of green tea waste silage in the diet on a DM basis, 5.4 kg DMI, 2.09% total catechins in green tea waste silage, and 422 kg average BW. The ingestion of 127 mg total catechins is comparable to that of one cup of green tea drunk daily by a human (Nakagawa et al., 1999). If we assume that the human BW is 60 kg, then 53.5 mg/kg of total catechins ingestion in this study would be equivalent to 3.210 mg intake by a human. This dosage is equivalent to a daily intake of over 24 cups of green tea. Green tea waste silage which contains 96.9 mg tocopherol/100 g DM (DMI; 5.4 kg/d) can be estimated to provide an intake of 5,233 IU (mg) daily of vitamin E by the steers in this study, because the silage contained fat-soluble vitamins that had not been extracted by hot water. Although the concentration of plasma vitamin A was not affected by feeding the steers green tea waste silage, the concentration of plasma vitamin E significantly increased both in the tea and PEG groups before, and 3 h after, the morning feeding in this study. Supplementation of high levels of vitamin E (at least 1,000 IU per day) during the dry period and early lactation can reduce the incidence of mastitis, possibly because of an increase in immune system activity and function, but supplementation appears to confer little benefit on other infectious diseases (Allison and Laven, 2000). The oxidative status is imbalanced during the early lactation phase in Holstein cows (Miller et al., 1993a; Bernabucci et al., 2002). Summer conditions capable of producing moderate heat stress cause oxidative stress in transition dairy cows (Bernabucci et al., 2002). The clinical mastitis risk could be controlled by supplementation with vitamins A, D and E in the late gestation period, because of the potential relationship between oxidative stress and mastitis

(Barnouin and Chassagne, 1998). From this evidence, the health and fertility of cattle are believed to be enhanced by consuming green tea waste, which contains high levels of catechins and vitamin E and actually increases plasma antioxidative activity and the concentration of vitamin E, as observed in this study.

IMPLICATIONS

To our knowledge, no study has investigated the effects of waste from green tea beverage manufacturing on ruminal fermentation and blood components. The results from the present study suggest that feeding diets containing 20% of the dietary DM as green tea waste silage to Holstein steers had no negative impact on ruminal fermentation, and increased plasma antioxidative activity and the concentration of vitamin E. It is most likely that high-producing lactating cows are under serious oxidative stress, because oxygen uptake by lactating cows is 2 to 3 times greater than by non-lactating cows. Therefore, we believe that adding green tea waste silage to the diets of cattle would benefit their health, because high levels of antioxidants and vitamin E can protect cells and tissues from oxidative damage. Feeding green tea waste to cattle would not prevent consumers in Asia from buying their milk or meat, because green tea is one of the most familiar beverages in Asia. Furthermore, green tea waste is not only low cost but also rich in protein, catechins and vitamins A and E, and thus would benefit both cattle and farmers.

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