The Effects of Three Herbs as Feed Supplements on Blood Metabolites, Hormones, Antioxidant Activity, IgG Concentration, and Ruminal Fermentation in Holstein Steers

K. Hosoda*, K. Kuramoto[1, 2], B. Eruden, T. Nishida[3] and S. Shioya

Department of Animal Feeding and Management, National Institute of Livestock and Grassland Science

Nasushiobara, Tochigi 329-2793, Japan

ABSTRACT : The aim of this study was to investigate the effects of three herb supplementations on blood metabolites, hormones, antioxidant activity, immunoglobulin (Ig) G concentration, and ruminal fermentation in steers. Four Holstein steers in a 4×4 Latin square design received four herb treatments. The treatments consisted of the steers' regular diets with addition of: 1) nothing (control), 2) peppermint, 3) clove, and 4) lemongrass at 5% of the diet (DM basis). Clove supplementation increased the plasma concentration of cholesterol by about 10% (from 79 to 87 mg/dl). Peppermint and lemongrass feeding resulted in an increase in the concentrations of plasma urea nitrogen (from 5.9 to 6.9 and 6.4 mg/dl, respectively). The three herb treatments had no effect on other metabolites and hormones. Steers receiving clove supplementation showed a higher plasma antioxidant activity. The three herb treatments caused lower concentrations of IgG in the blood. Peppermint and lemongrass feedings increased, and clove feeding decreased ruminal concentrations of ammonia. There were no significant differences in VFA concentrations among herbal treatments, except for the decrease in propionate concentration in steers receiving clove treatment. This study suggested that clove feeding changed cholesterol metabolism and increased antioxidant activity in plasma, and feeding of three herbs affected immunity system and ruminal fermentation in steers. (*Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 1 : 35-41*)

INTRODUCTION

It has been reported that when dried herbs were fed to lactating dairy cows, the characteristic smell of cow milk was suppressed due to the transmission of components peculiar to such herbs into the cows' milk (Ando et al., 2001). This study demonstrated that milk flavor could be controlled by the use of herb(s) as a feed supplement for dairy cows. Moreover, herb feeding to cattle is possibly beneficial for the welfare of the animals and decreases stress for them, as herbs and their preparations have been commonly used in aromatherapy for humans.

We previously investigated the suitability of various herbs as supplements to cattle feed. As a result, *Mentha×piperita* L. (peppermint), *Syzygium aromaticum* (clove), and *Cymbopogon citratus* (lemongrass) were edible as feed supplements by cattle (unpublished result). However, these herb preparations have been reported to possess functional activities *in vitro* and in mongastric animal studies. It is reported that peppermint oil exhibits

* Corresponding Author: K. Hosoda. Tel: +81-287-37-7806, Fax: +81-287-36-6629, E-mail: kenhoso@affrc.go.jp
[1] College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa, 252-8510, Japan.
[2] Dairy Research Farm, Zenrakuren, Yabuki, Fukushima, 969-0223, Japan.
[3] Japan International Research Center for Agricultural Sciences, Ibaraki 305-8686, Japan.
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antimicrobial activity (Pattnaik et al., 1996; Montes-Belmont and Carvajal, 1998; Imai et al., 2001) and inhibitory activity on motility of the gastro-intestinal tract in monogastric animals (Duthie, 1981; Hawthorn et al., 1988; Hills and Aaronson, 1991), and has been used for the treatment of irritable bowel syndrome (Rees et al., 1979; Kline et al., 2001) and dyspepsia (May et al., 2000) in humans. Clove oil has been demonstrated to have antioxidant (Kim et al., 1994; Dragland et al., 2003; Yun et al., 2003), antimicrobial (Smith-Palmer et al., 1998; Dorman and Deans, 2000), choleretic (Yamahara et al., 1983), and insecticidal (Kim et al., 2003; Park and Shin, 2005) activities, and a relaxant effect on smooth muscle (Reiter and Brandt, 1985). In lemongrass, antibacterial (Dikshit and Husain, 1984; Valero and Salmeron, 2003), antioxidant (Cheel et al., 2005), and antinociceptive (Viana et al., 2000) activities have been studied. Additionally, these three herbs also have been used as traditional folk remedies (Chevallier, 1996; Bown, 1997).

In the previous study (Hosoda et al., 2005), it was found that the feeding of peppermint to lactating dairy cattle decreased nutrient digestibility, changed energy metabolism, and decreased methane production. However, few data are available that document the effects of feeding herbs to cattle on blood profiles and ruminal fermentation. Therefore, the aim of the present study was to determine the effects of the supplementation to the diet of Holstein steers with three herbs, i.e. peppermint, clove and lemongrass, on metabolites,

Table 1. Nutrient compositions of diet and herbs

	Diet	Peppermint	Clove	Lemongrass
Dry matter (DM, %)	88.0	83.6	71.8	86.0
Nutrient composition (% DM)				
Organic matter	95.0	87.4	92.3	91.7
Crude protein	9.7	24.3	8.1	10.6
Neutral detergent fiber	36.2	30.6	28.8	63.7
Acid detergent fiber	20.2	23.0	25.8	31.9
Ether extracts	2.7	3.6	7.0	5.1
Ash	5.0	12.6	7.7	8.3

hormones, antioxidant activity, immunoglobulin (Ig) G concentration in blood, and ruminal fermentation.

MATERIALS AND METHODS

Animals and experimental design

The experiment was conducted as a 4×4 Latin square design. Four Holstein steers with ruminal fistula [body weight, 420.1±60.7 kg (mean±SD)] were used and maintained in stalls. The animals received a diet to meet the total digestible nutrients requirements for maintenance of the Japanese Feeding Standard for Beef Cattle (Agriculture, Forestry and Fisheries Research Council Secretariat, 2000), and were allocated to one of the following herbal additives: 1) no supplement (control), 2) peppermint, 3) clove, or 4) lemongrass. The herbs, which were purchased from Kaneka Sun Spice Co. Ltd (Osaka, Japan), were given at 5% of the diet on a dry matter basis. Feed was offered in equal amounts twice daily at 09:00 and 18:00 h and water was provided *ad libitum*. Each herb was mixed with the feed and provided to the animals. The diet consisted of 50.0% timothy hay, 42.2% flaked corn, 8.0% soybean meal, 1.2% mineral mix, 0.3% vitamin mix, and 0.3% salt. The mineral mix contained 110 g of P, 220 g of Ca, and 100 g of Mg per kg. The vitamin mix contained 1200 mg of vitamin A, 10 mg of vitamin D, and 20,000 mg of DL-α-tocopherol acetate per kg. The nutrient compositions of diet and herbs were shown in Table 1. Ground peppermint leaf, ground clove bud, and 1 cm-cut lemongrass leaf were used. Each experimental period consisted of a 2-week adaptation phase followed by a phase for collecting samples. All animals received humane care as outlined in 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 (Curtis and Nimz, 1988).

Sample collections

Blood samples were collected from the jugular vein using the tubes with heparin or EDTA just before morning feeding, and the tubes were immediately placed in ice. The collected samples were centrifuged at 1,200×g for 15 min at 4°C, and thereafter plasma samples were stored at -80°C until analysis.

Ruminal fluid was collected via the fistula just before the morning meal. The pH of the ruminal fluids was immediately measured using a pH meter (D-24, HORIBA, Tokyo, Japan). Ruminal fluids were separated from the feed particles through four layers of gauze, and centrifuged at 1,200×g for 15 min. The supernatants were treated with a perchlorate solution to deproteinize and then stored at -20°C until the assay.

Sample analyses

Feed dry matter was measured by drying the sample at 100°C for 18 h. Feed samples for chemical analysis were air-dried at 60°C and ground with a 1-mm sieve prior to analysis. Crude protein, ether extract, crude ash (AOAC, 2000), neutral detergent fiber and acid detergent fiber (Van Soest et al., 1991) were determined. Organic matter was computed as dry matter minus crude ash.

Plasma concentrations of glucose, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglyceride, none esterified fatty acids (NEFA), urea nitrogen (UN), glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT) were measured by enzymatic methods and total protein was measured by biuret method using the commercial kits of Wako Pure Chemical (Osaka, Japan; L-Type Wako Glu2, L-Type Wako CHO·H, L-Type Wako HDL-C, L-Type Wako LDL-C, L-Type Wako TG·H, NEFA-HA test Wako, L-Type Wako UN, L-Type Wako GOT·J2, L-Type Wako GPT·J2, and TPII-HA test Wako, respectively) with the automatic spectrophotometer (Clinical Analyzer 7070, Hitachi, Tokyo, Japan). Insulin and glucagon concentrations in plasma were determined using the double-antibody radioimmunoassay kits (Insulin "Eiken", EIKEN CHEMICAL, Tokyo, Japan, and glucagon kit "Daiichi", TFB, Tokyo, Japan). Plasma antioxidant activity was measured by a commercially available kit according to the manufacturer's protocol (Total Antioxidant Status, Randox Laboratories, Co. Antrim, UK). Briefly, the standard and sample were mixed with chromogenic reagent, and were incubated with H_2O_2 for 3 min at 37°C. Thereafter, the optical density was measured for its absorbance at 600 nm using a spectrophotometer (U-2001, Hitachi). The plasma concentration of IgG was measured by sandwich ELISA kit

Table 2. Dry matter intake by steers fed a diet supplemented with herbs (g/day)

	Control	Peppermint	Clove	Lemongrass
Diet	2,352.5±207.1	2,352.5±207.1	2,352.5±207.1	2,352.5±207.1
Herb	0.0±0.0	117.0±10.3**	117.4±10.2**	117.2±10.5**
Total	2,352.5±207.1	2,469.5±217.5**	2,469.9±217.4**	2,469.8±217.7**

Treatment differences compared with control, ** p<0.05.

Table 3. Plasma concentrations of metabolites from steers fed a diet supplemented with herbs

	Control	Peppermint	Clove	Lemongrass
Glucose (mg/dl)	74.9±8.7	76.3±5.8	73.4±2.5	73.9±3.8
Total cholesterol (mg/dl)	79.1±12.3	76.0±14.4	87.5±15.0*	84.3±18.9
HDL-cholesterol (mg/dl)	50.4±8.2	49.5±10.2	55.9±8.9 **	54.0±10.0
LDL-cholesterol (mg/dl)	22.6±5.3	20.2±4.1	26.1±6.8*	24.1±8.9
Triglyceride (mg/dl)	28.0±6.8	29.2±7.4	28.7±7.5	27.7±4.9
NEFA (mEq/l)	0.21±0.22	0.16±0.09	0.17±0.11	0.11±0.06
Total protein (g/dl)	8.0±0.5	8.0±0.4	8.2±0.4	8.2±0.6
UN (mg/dl)	5.9±0.3	6.9±0.6**	6.0±1.0	6.4±0.7*
GOT (U/l)	48.9±6.9	51.7±9.8	53.7±7.2	59.1±21.6
GPT (U/l)	15.2±1.9	15.8±3.6	15.9±2.1	15.5±1.3

HDL-cholesterol: High density lipoprotein cholesterol; LDL-cholesterol: Low density lipoprotein cholesterol.
NEFA: None esterified fatty acids; UN: Urea nitrogen; GOT: Glutamic oxaloacetic transaminase; GPT: Glutamic pyruvic transaminase.
Treatment differences compared with control; * p<0.10, ** p<0.05.

Table 4. Hormone concentrations, Antioxidant activity, and immunoglobulin G concentration in plasma from steers fed a diet supplemented with herbs

	Control	Peppermint	Clove	Lemongrass
Insulin (µU/ml)	11.3±1.4	10.2±2.4	10.7±2.2	11.3±3.2
Glucagon (pg/ml)	117.9±83.0	110.0±85.8	120.4±94.8	109.0±75.6
Antioxdant activity (µmol/l)[1]	709.1±60.3	739.5±66.0	775.7±55.4*	701.3±66.5
Immunoglobulin G (mg/dl)	1533.7±328.4	1180.7±393.7**	1370.0±299.3*	1330.3±460.2**

[1] Converted value as 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.
Treatment differences compared with control; * p<0.10, ** p<0.05.

(CellTrend GmbH, Luckenwalde, Germany). All plasma assays were performed in a single assay except for antioxidant activity assay. The inter-assay CV of antioxidant activity assay was 5.2%, and the intra-assay CVs of all assays were less than 5.1%.

Deproteinized ruminal fluid samples were neutralized with potassium hydroxide solution and centrifuged at 400×g for 10 min. The supernatants were subjected to ammonia (Weatherburn, 1967) and VFA analyses by HPLC system reported previously (Hosoda et al., 2005).

Statistical analysis

Differences in the parameters among dietary treatments were analyzed by GLM of SAS followed by Duncan's multiple range test (SAS, 1988). The p<0.05 and p<0.10 between the control and herb treatments were considered to show statistical significance and tendency, respectively. Data were displayed as means with standard deviations.

RESULTS

Animals consumed all feed and herb supplements at all treatments and phases. The DMIs of the diet by steers were similar among treatments, whereas the DMIs of herb and total diet by steers in the control group were significantly lower (p<0.05) than those of steers receiving the herb treatments because of addition of herb (Table 2).

The effects of the herb treatments on plasma metabolites are shown in Table 3. The plasma concentration of total cholesterol from steers receiving the clove treatment tended to be higher (p<0.10) than that from steers in the control group. Steers offered a diet containing clove have significantly higher (p<0.05) concentration of HDL-cholesterol in plasma than that in the control group. LDL-cholesterol concentration in the clove treatment group also tended to be higher (p<0.10) than that in the control group. The plasma UN concentrations in the peppermint and lemongrass treatment groups were statistically higher (p<0.05 or p<0.10) than those in the control group. The concentrations of glucose, triglyceride, NEFA, total protein, GOT, and GPT in plasma were unaffected by the herb feeding treatments. There were no significant differences in the plasma concentrations of insulin and glucagon among treatments (Table 4). Clove feeding tended (p<0.10) to increase plasma antioxidant activity compared with the control group (Table 4). All herb ingestions resulted in

Table 5. Ruminal fermentation in steers fed a diet supplemented with herbs

	Control	Peppermint	Clove	Lemongrass
Ruminal pH	6.85±0.10	6.83±0.13	6.78±0.22	6.90±0.09
Ammonia (mg/dl)	5.0±2.1	6.7±2.6 **	3.2±0.4*	5.4±2.2**
VFA concentration (mM)				
Acetate	51.4±3.4	50.6±9.6	46.0±8.6	48.6±5.8
Propionate	16.6±1.2	15.0±2.7	13.4±1.1**	14.8±0.8
Butyrate	9.4±1.9	8.6±1.6	9.3±1.1	8.7±1.9
Total	80.8±3.6	77.4±14.3	71.9±9.1	74.9±7.9
Molar rate of VFA (%)				
Acetate	63.6±2.7	65.2±2.2	63.7±4.1	64.8±1.8
Propionate	20.5±1.7	19.4±2.1	18.9±2.2*	20.0±2.7
Butyrate	11.6±2.2	11.1±0.5	13.2±2.9	11.5±1.6
Acetate to propionate ratio	3.1±0.3	3.4±0.5	3.4±0.6	3.3±0.5

Treatment differences compared with control; * $p < 0.10$, ** $p < 0.05$.

statistically lower ($p < 0.05$ or $p < 0.10$) concentrations of IgG than that of the control group (Table 4).

Ruminal fermentation in steers receiving herb treatments is presented in Table 5. Ruminal pHs were unaffected by the dietary herb supplementations. Peppermint and lemongrass feeding significantly ($p < 0.05$) increased, and clove feeding tended ($p < 0.10$) to decrease the ammonia concentrations compared with those in the control group. There were no significant differences in VFA concentrations and molar rates of VFA among herbal treatments, except for the decrease in propionate concentration and propionate molar rate in steers receiving clove treatment ($p < 0.05$ and $p < 0.10$). Herb supplementations had no effects on acetate to propionate ratio.

DISCUSSION

The concentrations of metabolites, hormones, and IgG in plasma were examined in the preset study. This is because these are considered to be the indicators for investigating the effect of herb feeding on physiology in cattle, i.e. glucose, insulin, and glucagon for glucose metabolism, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, and NEFA for lipid metabolism, total protein and UN for nitrogen metabolism, GOT and GPT for liver function, IgG concentration for immunity.

In general, the total cholesterol concentration in blood can arise from either exogenous origins, namely meals and supplements, or endogenous origins. In the present study, the concentration of total cholesterol in the plasma from the clove treatment group tended to be higher than that of the control group and, as clove contains no cholesterol (Resources Council, Science and Technology Agency, Japan, 2000), steers receiving the clove treatment ingested the same amount of cholesterol as those in the control group. This indicates that the increased value of total cholesterol in the clove treatment group can be attributed to endogenous

origins. In a study with human subjects (Grundy and Denke, 1990), the concentration of total blood cholesterol was increased by dietary saturated fatty acids and decreased by polyunsaturated fatty acids, with the saturated fatty acids being twice as potent in terms of the induced magnitude of change in blood cholesterol concentration. Likewise, in cattle, those fatty acids can also regulate the lipid metabolism in the liver (Bauchart, 1993; Leplaix-Charlat et al., 1996). Clove (100g) contains only 5.4 and 7.1 g of saturated and polyunsaturated fatty acids (USDA, Agricultural Research Service, 2004) and with a dietary inclusion rate of 5%, clove contributed only a small fraction of the dietary fatty acid content. Therefore, we suggest that a specific component(s) in clove enhanced the production, metabolism, and/or circulation of total cholesterol. In addition, it has been reported in humans that increased concentrations of HDL-cholesterol are observed with a decreased in those of LDL-cholesterol in blood (Kris-Etherton and Yu, 1997). However, in the present study, clove feeding increased both plasma HDL- and LDL-cholesterol concentrations compared with those in the control group. This result indicates that clove feeding is likely to increase the amounts of lipoproteins *per se* rather than to change ratio of HDL- and LDL-cholesterol concentrations, and also supports our insistence that a specific component(s) in clove elevated circulating concentration of total cholesterol.

Urea is synthesized in the liver from ammonia absorbed from the rumen or gut, and so a UN concentration in blood is positively correlated with the ruminal concentration of ammonia (Lewis, 1957; Petit and Flipot, 1992; Davidson et al., 2003). Similar to those reports, the increases of plasma UN concentrations by peppermint and lemongrass feedings in the present study were probably caused by the differences of ruminal fermentation between the herb treatment groups and control group, because higher concentrations of ruminal ammonia in the peppermint and lemongrass treatment groups than in the control group were

concomitantly obtained.

It has been reported that the three herbs used in this study, i.e. peppermint, clove and lemongrass, have pharmacological activity (Yamahara et al., 1983; Viana et al., 2000; Inoue et al., 2001). The ingestion of these herbs possibly decreases the function of organs. In the present study, the herb-feeding treatments resulted in no decreases in the values of plasma metabolites, enzymes or hormones compared with the control group, although elevated cholesterol and UN concentrations were seen. These findings suggest that herb feeding does not appear to have an inhibitory effect on the function of organs associated with plasma substances examined in this study. However, long-term (more than 2 weeks) effects of herb feeding on the function of organs in cattle have never been studied.

The dietary supplementations of antioxidants such as selenium or vitamin E have been reported to decrease the risks of retained placenta, metritis, and clinical mastitis after delivery in cattle (Brzezinska-Slebodzinska et al., 1994; Erskine et al., 1997; Weiss et al., 1997). These previous studies suggest that antioxidant ingestion should bring health benefits in cattle. Clove, which was used in this study, is well known as a source of potent antioxidants (Kim et al., 1994; Dragland et al., 2003; Yun et al., 2003). As shown in Table 4, only clove feeding among the three herb treatments increased the antioxidant activity of the steers' plasma compared with the control group, which indicates that the antioxidant effect caused by clove feeding was not transient and was maintained for approximately a half day. Therefore, clove is likely excellent among three herbs as a source of antioxidants for cattle, and its ingestion may contribute to cattle health.

It has been reported that immune response and immunity status were affected by the ingestion of antioxidant substances and oxidative stress (Grasso et al., 1990; Politis et al., 1995; Miyazaki et al., 2001). Stabel et al. (1989) demonstrated that the administration of selenium as an antioxidant source to calves inoculated with *Pasteurella hemolytica* resulted in a decrease in anti-*P. hemolytica* titers. Their report is likely to be consistent with our result that the steers receiving the clove supplement, which is a potent antioxidant, carried a lower concentration of IgG. In contrast, Chatterjee et al. (2003) reported that vitamin E supplementation as an antioxidant source increased both the antioxidant activity and IgG concentration of plasma in periparturient cows. Moreover, although peppermint and lemongrass feeding to steers decreased the plasma concentration of IgG, it did not change their antioxidant activity compared with the control group. At least, these results indicate that herb feeding could affect the immune system in steers.

The results from the present study showed that peppermint and lemongrass feeding increased, and clove feeding decreased ruminal concentrations of ammonia. Ruminal ammonia concentration has been reflected in the balance of nitrogen and energy supplies into the rumen, which is associated with microbial activity (McDonald et al., 2002). The ruminal alteration of ammonia concentration in steers receiving herb treatments is possibly explained by such supply balance of nitrogen and energy. However, as additive rates of herbs were only 5% of diet and herbs contain nitrogen and energy, it is probably difficult to solve the reason for ammonia alteration in the present study. As to ruminal VFA concentration, it is generally known that fibrous feed causes a rise in acetate proportion, whereas the addition of concentrate to diet leads to an increase in propionate proportion at the cost of acetate (McDonald et al., 2002). However, clove supplementation resulted in the reduction of propionate concentration and molar rate without the change of those of acetate. While, in the previous study (Hosoda et al., 2005), we demonstrated that the feeding of a diet with peppermint supplementation at 5% of the diet reduced nutrient digestibility and changed energy partition in lactating cattle, which indicates that peppermint feeding has an effect on the digestion system in cattle. Taken together, a specific component(s) in herbs is likely to be involved in the ruminal alternation of ammonia and VFA concentration in steers fed herbs.

In conclusion, our results showed that the supplementation of three herbs to diet affected nutrient metabolism, antioxidant activity, immune system, and ruminal fermentation in steers. Scientific studies that report the effect of the feeding of herbs, such as peppermint, clove, and lemongrass, to ruminants is limited. In addition, the regulatory mechanisms through which herb feeding affected those blood values and ruminal fermentation parameters in the present study is far from being understood, and further study is required.

ACKNOWLEDGEMENTS

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