OPEN ACCESS Asian Journal of Animal and Veterinary Advances

ISSN 1683-9919
DOI: 10.3923/ajava.2018.377.382

Research Article
Effects of Coffee Ground Silage Feeding in Reducing Somatic Cell Count in Bovine Subclinical Mastitis Milk

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Abstract

Background and Objective: Bovine subclinical mastitis increases somatic cell count (SCC) without showing clinical symptoms, thus resulting in substantial economic losses to dairy farms. Numerous attempts to reduce SCC without using antibiotics have been reported but they have yet to be applied widely. This study investigated whether SCC can be reduced by feeding coffee ground silage containing large amounts of polyphenol, which is an inexpensive antioxidant to cows with subclinical mastitis. **Materials and Methods:** In cows with subclinical mastitis, the coffee ground silage feeding group was fed by adding 1.5 kg day^{-1} to normal feed and feed in the control group was not supplemented. Blood and milk were collected every week, the SCC in the quarter milk was measured and the oxygen radical absorbance capacity (ORAC) values in the blood and milk and the thiobarbituric acid reactive substances (TBARS) value in milk were measured. In order to investigate the immune kinetics, leukocyte subpopulation analysis was performed for 7 days after feeding. **Results:** Although the SCC did not show a significant decrease in the non-feeding group (n = 9), in the coffee ground silage feeding group (n = 11), it was decreased significantly ($p<0.01$) at the 5th week. Furthermore, ORAC values were significantly higher ($p<0.05$) in the coffee ground silage group (n = 5) than in the non-feeding group (n = 6), the TBARS value was significantly lower ($p<0.01$). In addition, bulk milk SCC after feeding was also observed in 150 milking cattle in the summer season under heat stress. Furthermore, in the subpopulation of leukocytes after 7 days of feeding, there was a significant increase in CD4+cells and CD14+MHC class II+cells on day 1 after feeding. **Conclusion:** These results suggest that feeding of coffee ground silage to cows with subclinical mastitis increased the antioxidant activity and immune activity, leading to a decrease SCC in milk.

Key words: Antioxidant activity, bovine mastitis, subclinical, coffee ground silage, polyphenol, somatic cell count and immune activity

Citation: Kazuhiro Kawai, Kana Kuruhara, Yutaka Matano, Kiyoshi Akiyama, Shinji Hashimura, Sachi Tanaka, Yoshio Kiku, Aiko Watanabe and Yasunori Shinozuka, 2018. Effects of coffee ground silage feeding in reducing somatic cell count in bovine subclinical mastitis milk. Asian J. Anim. Vet. Adv., 13: 377-382.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bovine mastitis is an important disease that causes substantial economic losses to dairy farms[1]. In particular, subclinical mastitis increases SCC in milk and reduces the amount of milk produced, mainly due to inflammation of the mammary gland[1-3]. However, most of the cases are not actually treated with antibiotics and they reduce shipping milk volumes, which is a major factor impeding the improvement of milk quality. Therefore, methods for reducing the SCC in milk without performing antibiotic therapy have been sought. It has been reported that various immune cells are activated by administering antioxidant substances such as vitamin E and selenium to dairy cattle[4,5] to suppress the damage of mammary epithelial cells due to active oxygen produced by inflammation and they also reduce SCC and prevent mastitis[6-9]. On the other hand, it is known that coffee grounds contain many polyphenols showing antioxidant activity at 2 to 4 times the levels seen in green tea, black tea and cocoa[10]. It has also been reported that polyphenols show a positive correlation with Cu reducing ability, DPPH radical scavenging ability and superoxide scavenging activity[10,11].

Polyphenols from coffee beans are chlorogenic acids, such as caffeic acid and quinic acid and these are known to be present in coffee grounds[12]. However, coffee grounds have poor palatability to cattle and are difficult to use as feed and they are therefore disposed of as industrial waste.

The purpose of this study was to investigate the efficacy of coffee ground silage feeding on SCC reduction in cows with subclinical mastitis and it was clarified that coffee ground silage feeding decreases somatic cell count of subclinical mastitis cows.

MATERIALS AND METHODS

This study was conducted based on the ethical standards of the Animal Experiment Committee of Azabu University.

Coffee ground silage: Used coffee grounds were packed in a plastic bag at a coffee mass retailer, added vinegar and sealed in the store and transported to the factory. In the silage processing plant, silage was prepared by adding a fermentation enzyme while adjusting the moisture and the silage was sealed with vinyl and put in a flexible container bag and transported to the farm in units of 400 kg of 10 pieces. The silage produced had a moisture of 59.5%, pH of 4.2 and the Total Digestive Nutrients and Crude Protein content of the actual products were 29 and 8.5% on a dry matter basis, respectively.

Measurement of quarter milk somatic cell count: From 2013-2014, measurement of SCC was conducted on 44 quarters from 20 subclinical mastitis cows with SCC of 200,000 cells mL^{-1} or more bred in Kanagawa Prefecture. Cows were bred in tie stall housing with free access to drinking water. Eleven of the cows (22 quarters) received 1.5 kg day^{-1} of coffee ground silage mixed into their usual feed and the remaining 9 cows (22 quarters) were assigned to the control group with no supplemental coffee ground silage. Milk samples were collected before and at 1-4 weeks after introducing the feed and SCC in milk was measured using a DeLaval cell counter (DeLaval International AB, Tumba, Sweden) according to method of Kawai *et al.*[13].

Measurement of antioxidant activity: During the heat stress period of the same study period (2013-2014), antioxidant activity was measured for 11 cows randomly selected from the above herd in Kanagawa Prefecture. Five of the cows received coffee ground silage and the remaining 6 cows were assigned to the control group with no coffee ground silage. Blood and milk samples were collected before and at 1-5 weeks after introducing the feed, antioxidant activity in the form of ORAC was measured in blood and milk and lipid peroxidation in the form of TBARS was measured in blood. For measurement of the ORAC value, the OxiSelect™ ORAC activity assay kit (Cosmo Bio Inc., Tokyo, Japan) was used. For measurement of TBARS values, the OxiSelect™ TBARS assay kit (Cell Biolabs Inc., San Diego, CA) was used.

Measurement of bulk milk somatic cell count: Coffee ground silage was given at 1.5 kg day^{-1} during the summer season of 2015, in a herd of 150 milking cows in Aichi Prefecture. Milk samples were collected every 10 days and changes in the average bulk SCC after temporarily discontinuing feeding and after restarting feeding were compared. Furthermore, the daily milk yield and the maximum temperature and the lowest temperature at the time of sampling were recorded to observe the relationship under heat stress in the summer season.

Influence on immune cells in milk: Twelve subclinical mastitis cows with SCC of 200,000 cells mL^{-1} or more bred in Nagano Prefecture were tested for the influence on immunity in milk in 2015. Cows were bred in tie stall housing with free access to drinking water. Coffee ground silage was fed to 7 cows by mixing 1.5 kg day^{-1} into their usual TMR feed and the other 5 acted as the non-feeding control group. Milk samples were collected before feeding and on days 1, 4 and 7 after feeding and were stored at 4°C. Leukocyte subpopulations in

milk were analyzed using a flow cytometer (BD FACSCalibur, Becton Dickinson and Company, Franklin Lakes, NJ) within an hour from collection.

Statistical analysis: Comparisons of SCC were performed using the Wilcoxon signed-rank test. ORAC activity values and TBARS values were analyzed using the Wilcoxon rank sum test. The influence on immunocompetence was analyzed using the Wilcoxon rank sum test and Wilcoxon signed-rank test. Statistical analyses were conducted using IBM SPSS Statistics 22 (IBM Co., North Castle, NY). The $p<0.05$ was considered to be significant.

RESULTS

Unlike the control group, which showed no significant decreases in SCC in milk, the cell count at weeks 2, 3 and 4 in the coffee ground silage group had decreased significantly ($p<0.01$) from baseline (Fig. 1). Blood and milk ORAC values were also significantly higher ($p<0.05$) at weeks 3, 4 and 5 in the coffee ground silage group when compared to the control group (Fig. 2) and blood TBARS values at week 5 were significantly lower ($p<0.01$) in the coffee ground silage group than in the control group (Fig. 3).

Furthermore, in cases similarly fed in the summer season, the bulk milk SCC increased after temporarily discontinuing feeding and bulk milk SCC decreased after restarting feeding (Fig. 4).

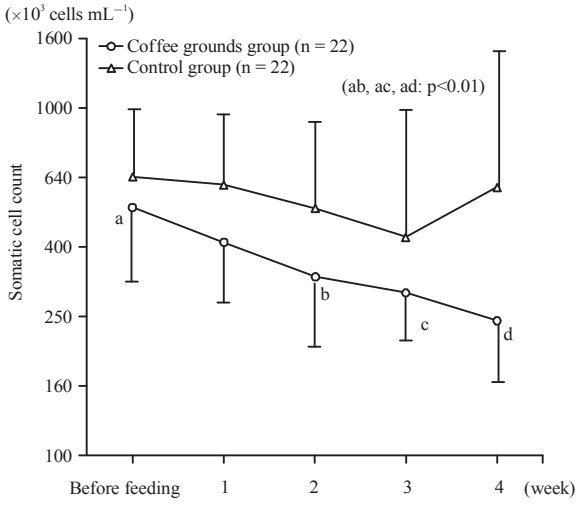

Fig. 1: Changes in mean SCC after feeding of coffee ground silage

SCC at weeks 2, 3 and 4 in the coffee ground silage group had decreased significantly ($p<0.01$) from baseline

Analysis of the subpopulation of leukocytes in milk up to the seventh day of coffee ground silage feeding revealed a significant increase in CD4+cells and CD14+MHC class II+cells on the first day of feeding when compared to before feeding in the coffee ground silage group (Fig. 5 and 6).

DISCUSSION

In this study, silaging coffee grounds and improving palatability enabled cows to eat and realized that the high

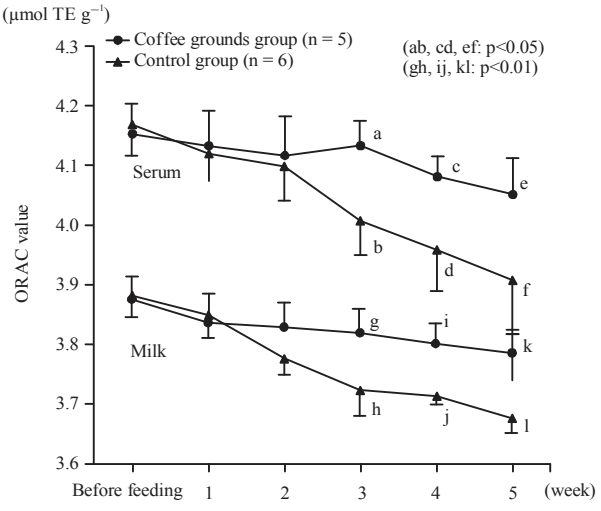

Fig. 2: Changes in mean ORAC values after feeding of coffee ground silage

Blood and milk ORAC values were also significantly higher ($p<0.05$) at weeks 3, 4 and 5 in the coffee ground silage group when compared to the control group

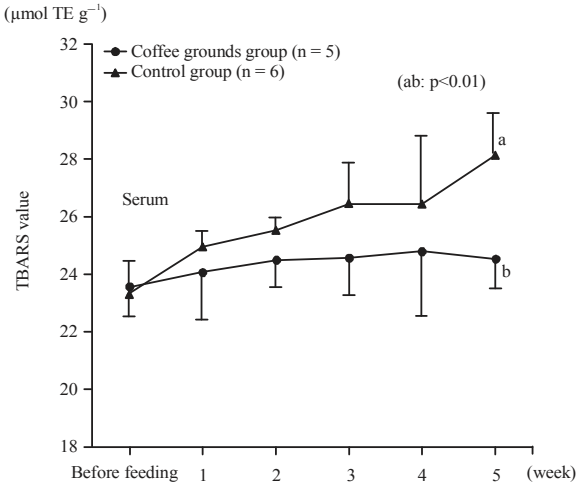

Fig. 3: Changes in mean TBARS values after feeding of coffee ground silage

Blood TBARS values at week 5 were significantly lower ($p<0.01$) in the coffee ground silage group than in the control group

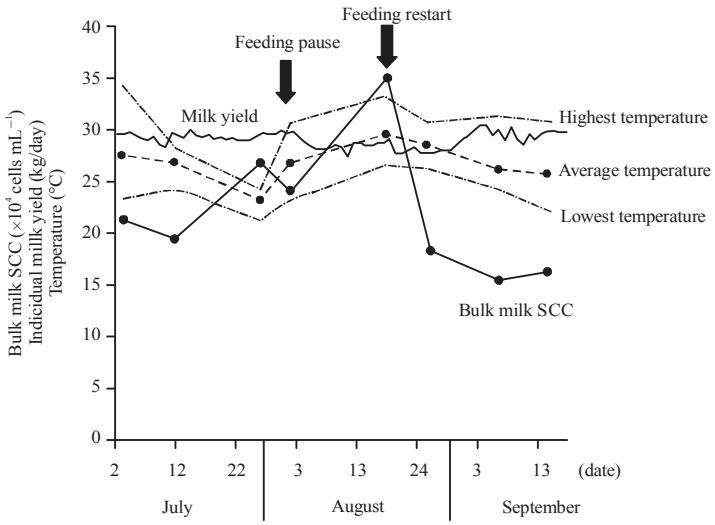

Fig. 4: Changes in bulk milk SCC and individual milk yield after feeding of coffee ground silage

When we fed coffee ground silage daily for the summer season, the bulk milk SCC increased after temporarily discontinuing feeding and decreased after restarting feeding

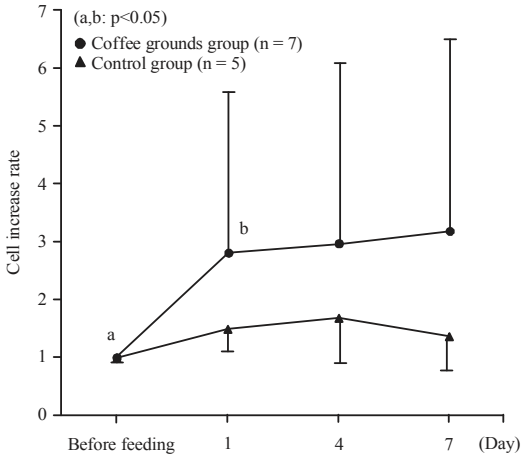

Fig. 5: Changes in CD4+cells in milk after feeding of coffee ground silage

Analysis of the subpopulation of leukocytes in milk up to the seventh day of coffee ground silage feeding revealed a significant increase ($p<0.05$) in CD4+cells on the first day of feeding when compared to before feeding

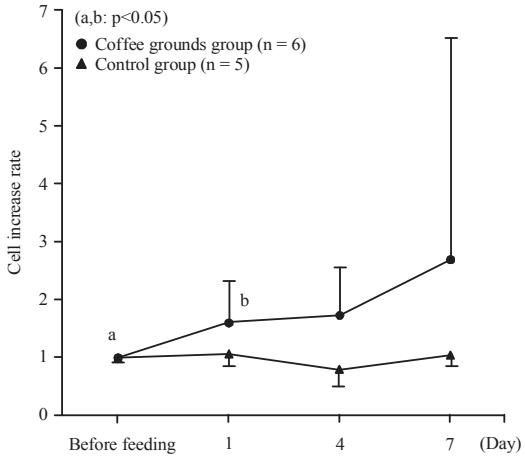

Fig. 6: Changes in CD14+MHC class II+cells in milk after feeding of coffee ground silage

Analysis of the subpopulation of leukocytes in milk up to the seventh day of coffee ground silage feeding revealed a significant increase ($p<0.05$) in CD14+MHC class II+cells on the first day of feeding when compared to before feeding

antioxidant effect originally possessed by coffee grounds can be used for cows. It also raises the antioxidant action not only in the blood but also in milk, which has greatly contributed to the prevention and treatment of subclinical mastitis.

Coffee grounds contain many polyphenols[10,11] and it has been reported that polyphenols have Cu reducing ability, DPPH radical scavenging ability and superoxide scavenging activity[10,11] and that chlorogenic acids such as caffeic acid and quinic acid, which are polyphenols are abundantly present in coffee grounds[12]. Therefore, the antioxidant effects of coffee ground silage being administered as cattle feed is expected. Caffeic acid and ferulic acid present in the coffee grounds are absorbed mainly into the body from the apical side of the small intestine and enter the blood[14-16]. Therefore, in this study, polyphenol was absorbed by the small intestine from coffee ground silage, thereby increasing the antioxidant activity in blood and milk and it was thought that lipid peroxidation decreased.

Furthermore, in the summer season with heat stress, the transition of bulk milk SCC was evaluated. When coffee

ground silage feeding was stopped, the number of somatic cells increased greatly, while restarting feeding reduced the SCC. It is known that as the SCC increases, milk yield loss increases[1]. It was confirmed that milk yield decreased as SCC increased and milk yield increased as SCC decreased. These results suggested a great economic effect even in the summer when heat stress is large.

In the subpopulation of leukocytes in milk up to day 7 of coffee ground silage feeding, the coffee ground silage group showed a significant increase in CD4+cells and CD14+MHC class II+cells on day 1 of feeding, as compared to before feeding, thus indicating an increase in helper T cells and macrophages. This suggested the induction of cellular immunity in the udder due to feeding of coffee ground silage. The immunopotentiating effects of coffee beans were supported by several reports[17-19]. However, further investigation is necessary to clarify the influence of coffee ground silage on immunocompetence. Feeding of coffee ground silage to cows with subclinical mastitis appears to result in activation of immune kinetics accompanying the increase in antioxidant activity and the SCC in milk decreased. From the results of this study, coffee ground silage feeding appeared to take at least 2 weeks to exert sufficient effects on reducing the SCC. However, because it is reasonable than vitamin E and selenium, it was seemed to be useful for achieving high quality milk production.

CONCLUSION

These results showed that feeding of coffee ground silage to dairy cattle promotes antioxidant activity in blood and milk and reduces the SCC. Furthermore, as a decrease in bulk milk SCC was also observed, coffee ground silage was considered to greatly contribute to the production of high-quality milk.

SIGNIFICANCE STATEMENT

This study showed that feeding coffee ground silage to dairy cows increased antioxidant levels in blood and milk, activated immunity and decreased somatic cell count in milk. This also greatly contributed to mastitis control. This research is the first to show that converted coffee grounds, which are generally considered to be an industrial waste product of the food and drink industry, may be a valuable feed supplement for dairy cows.

ACKNOWLEDGMENT

We are grateful to Starbucks Coffee Japan for providing the coffee grounds used in this study.

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