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REVIEW

Effects of Whole Crop Rice Silage on Meat Quality and Adipokine Gene Expression in Fattening *Wagyu* Cattle

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Abstract

The objective of this study was to investigate the effects of whole crop rice silage (WCRS) feeding on the lipid stability of beef and fat depot-specific adipokine gene expression patterns in *Wagyu* cattle. *Wagyu* steers were fed a control diet (CT, n=4) or a high-vitamin diet containing WCRS (HV, n=4) from 10 to 30 months of age. The α-tocopherol concentration of the longissimus muscle was significantly higher in the HV group than in the CT group. The percentage of metmyoglobin in the longissimus muscle was significantly lower in the HV group than in the CT group. The 2-Thiobarbituric acid-reactive substance values of the longissimus muscle were also significantly lower in the HV group than in the CT group. The plasma 8-isoprostane concentration, which is a biomarker of oxidative stress, was significantly lower in the HV group than in the CT group. The expression of adipokine genes (VEGF, FGF-2, and leptin) in mesenteric adipose tissue was significantly lower in the HV group than in the CT group. These results suggest that feeding beef cattle on WCRS improves both the quality of meat and the oxidative stress status.

Discipline: Animal industry
Additional key words: α-tocopherol, adipocyte

Introduction

Increasing the production of self-supplied feed to secure food supply capability is an important political issue in Japan. Recently, whole crop rice silage (WCRS) has been regarded as an important source of self-supplied feed in Japan. In 2015, the yield of WCRS in Japan was estimated to have reached an all-time high, due to increased cropping acreage.

The forage characteristics of WCRS have been investigated. The silicic acid content of WCRS tends to be higher than that in grass hay, while the crude ash of WCRS accounts for about 13% (NARO 2009). WCRS at the yellow ripe stage contained about 6-7% crude protein (CP), which is similar to that of whole crop barley silage (NARO 2009, Yamada et al. 2015). The percentage of total digestible nutrients (TDN) of WCRS at the yellow ripe stage is about 55% (NARO 2009). In particular, WCRS characteristically contains a high amount of α-tocopherol (NARO 2008). A liposoluble vitamin, α-tocopherol is also a potent antioxidant. WCRS at the yellow ripe stage contains about 150 mg/kg of α-tocopherol (Yamada et al. 2012). Dietary supplementation with α-tocopherol acetate has been shown to suppress lipid oxidation and improve the meat quality of beef cattle (Arnold et al. 1993, Faustman et al. 1998). Therefore, feeding WCRS to fattening cattle may improve meat quality by increasing the α-tocopherol concentration within skeletal muscles; however, the effects of WCRS on the meat quality of beef cattle still remain unclear. Obesity is characterized by increased oxidative stress with chronic inflammation in adipose tissue (Araki et al. 2010). Adipocyte secretes a large number of adipokines, the secretary patterns of which are affected by the state of obesity (Funahashi et al. 1999). However, the effects of dietary α-tocopherol on the expression of adipokine genes in fattening cattle have not been investigated. In this review, we focus on the effects of WCRS on meat quality and adipokine gene expression in *Wagyu* fattening cattle.

Experimental design

The experimental design was described in detail in our previous reports (Yamada et al. 2012, Yamada et al. 2013). In brief, at 10 months of age, *Wagyu* steers were divided into two groups. (1) The control group (CT, n=4) was fed concentrate (88% total digestible nutrients (TDN),

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2 mg/kg α-tocopherol)) and orchard grass hay (56% TDN, 3 mg/kg α-tocopherol)) during the experimental periods (from 10 to 30 months). (2) The high-vitamin diet group (HV, n=4) was fed total mixed rations (TMR) containing WCRS (Hamasari at the yellow ripe stage), which consisted of 72% TDN and 60 mg/kg of α-tocopherol (fed from 10 to 20 months), and 77% TDN and 22 mg/kg α-tocopherol (fed from 21 to 30 months). At 30 months of age, blood samples were collected, and the plasma levels of 8-isoprostane were measured using an ELISA kit (Yamada et al. 2013). At 30 months of age, the steers were slaughtered, and the longissimus muscle, subcutaneous adipose tissue, and mesenteric adipose tissue were sampled. The α-tocopherol concentrations, metmyoglobin (Mb) percentages, and values of 2-thiobarbituric acid-reactive substances (TBARS) in the longissimus muscle were measured as previously described (Yamada et al. 2012). Adipokine gene expressions in subcutaneous and mesenteric adipose tissue were analyzed by real-time PCR (Yamada et al. 2013).

Effects of WCRS on meat quality

The growth performance and carcass characteristics of *Wagyu* steers in this experiment were described in our previous reports (Yamada et al. 2012, Yamada et al. 2013). In brief, there were no significant differences in the final body weight and daily weight gain between the groups. In addition, there were no significant differences in carcass composition (separable bone, muscle, and adipose tissue) between the groups.

Previous reports have indicated that supplementing beef cattle with α-tocopherol acetate effectively extends the stability of color and lipids in meat (Arnold et al. 1993, Faustman et al. 1998). To prevent metmyoglobin formation and lipid oxidation, approximately 3 mg/kg of α-tocopherol should be accumulated within skeletal muscle (Mitsumoto et al. 1991, Arnold et al. 1993). In this study, the α-tocopherol concentration in the longissimus muscle was significantly higher in the HV group than in the CT group (Fig. 1). The results also showed that the α-tocopherol concentration in the longissimus muscle with WCRS feed was more than 3 mg/kg. These results suggest that beef from WCRS-fed cattle prevent metmyoglobin formation and lipid oxidation.

Meat color is an important factor that consumers use to judge meat quality. Oxidation causes meat color to change from bright red to brown. Oxymyoglobin is the bright red pigment observed on the surface of fresh meat. Oxidation causes oxymyoglobin to give way to metmyoglobin, which turns the meat surface brown (Liu et al. 1995). When the Mb % of meat becomes greater than 40%, consumers no longer consider the meat to be fresh (Greene et al. 1971). Dietary supplementation with α-tocopherol acetate suppresses the increased Mb % in beef (Liu et al. 1995, Faustman et al. 1998). In this study, we showed that the Mb % of the longissimus muscle was significantly lower in the HV group than in the CT group on day 7 and day 13 (Fig. 2). We also showed that the Mb % of the longissimus muscle on day 7 was under 40% in the HV group. These results suggest that WCRS feeding can be expected to cause an adequate amount of α-tocopherol to accumulate within skeletal muscle, thereby stabilizing meat color.

Lipid oxidation is also a major factor of deterioration in meat quality. The products of fatty acid oxidation produce off-flavors collectively described as a “rancid flavor” (Campo et al. 2006). The TBARS value increases with the progression of meat oxidation (Liu et al. 1995, Campo et al. 2006). TBARS value > 1 is usually associated with a rancid flavor (Jayasingh et al. 2002). Previous reports have indicated that dietary supplementation with α-tocopherol acetate suppresses an increase in the TBARS value of beef (Arnold et al. 1993, Liu et al. 1995). We showed that the TBARS value in the longissimus muscle of cattle was

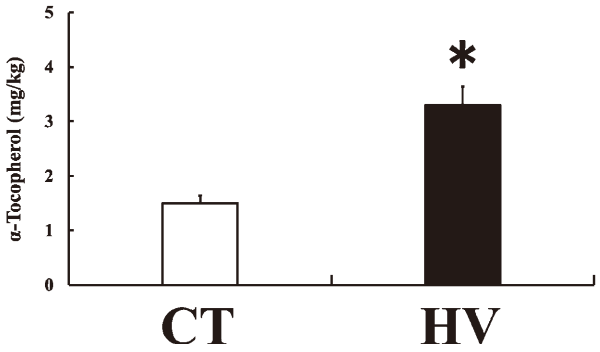

Fig. 1. α-tocopherol concentration in the longissimus muscle of *Wagyu* steers fed a control (CT) or high-vitamin (HV) diet. The data represent the means ± S.D. * P < 0.05. Based on data from Yamada et al. (2012).

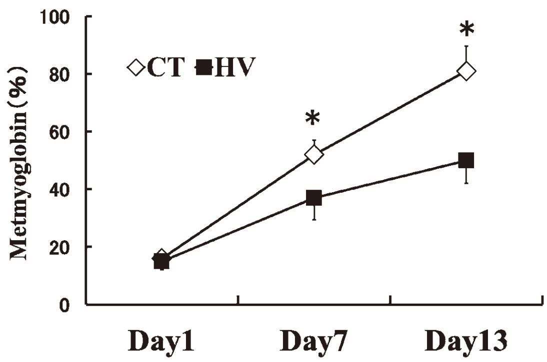

Fig. 2. Metmyoglobin formation (Mb %) in the longissimus muscle of *Wagyu* steers fed a control (CT) or high-vitamin (HV) diet. The data represent the means ± S.D. * Indicates a significant difference within each time point (P < 0.05). Based on data from Yamada et al. (2012).

significantly lower in the HV group than in the CT group on day 7 and day 13 (Fig. 3). We also showed that the TBARS value of the longissimus muscle on day 7 and day 13 was < 1 in the HV group. These results suggest that WCRS feeding suppress the production of off-flavors in beef.

Effects of WCRS on adipokine gene expression

Obesity increases the plasma levels of oxidative stress biomarkers, such as 8-isoprostane. A plasma concentration of 8-isoprostane reflects oxidative stress in obesity (Urakawa et al. 2003, Araki et al. 2010). *Wagyu* are conventionally fed a low β-carotene diet to improve the accumulation of intramuscular fat (Oka et al. 1998). Irie et al. (2006) reported low concentrations of α-tocopherol and β-carotene in the conventional fattening diet, and that *Wagyu* cattle raised using the conventional feeding system have low levels of carcass tissue α-tocopherol. Previous reports indicated that dietary supplementation with α-tocopherol reduced the plasma 8-isoprostane concentrations with the reduction of oxidative stress in obese humans (Laight et al. 1999, Ward et al. 2007). In this study, the plasma 8-isoprostane concentrations were significantly lower in the HV group than in the CT group (Fig. 4). These results suggest that *Wagyu* fattening cattle receiving low-vitamin diets are exposed to oxidative stress. These results also suggest that the feeding of WCRS, which contains a higher concentration of α-tocopherol, reduced oxidative stress in fattening *Wagyu* cattle.

Adipocyte secretes many adipokines, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2) (Gabrielsson et al. 2002, Miyazawa-Hoshimoto et al. 2005). Oxidative stress stimulates the expression of VEGF and FGF-2 genes in adipocytes (Lolmede et al. 2003). Leptin, the major adipokine secreted from hypertrophic adipocytes, induces oxidative stress, and its concentration in plasma has a positive correlation with oxidative stress levels (Nakanishi et al. 2005). We have shown that the expression of adipokine genes (VEGF, FGF-2, and leptin) in the mesenteric adipose tissue was significantly lower in the HV group than in the CT group (Fig. 5B). In contrast, there were no differences in adipokine gene expression levels in subcutaneous adipose tissue between the groups (Fig. 5A). In humans, higher oxidative stress conditions stimulate the secretion of adipokine (Kelly et al. 2006). In addition, oxidative stress is strongly associated with visceral rather than subcutaneous adiposity (Araki et al. 2010). These results suggest that the dietary intake of WCRS affects the expression pattern of adipokine genes in a fat depot-specific manner.

Conclusion

We have shown that WCRS feeding promotes the accumulation of α-tocopherol within skeletal muscle. The increased concentration of α-tocopherol in beef stabilizes meat color and suppresses lipid oxidation. We have also shown that the intake of WCRS reduces oxidative stress with the reduction of adipokine gene expression in mesenteric adipose tissue. These results suggest that feeding beef cattle with WCRS improves both the quality of meat and the oxidative stress status.

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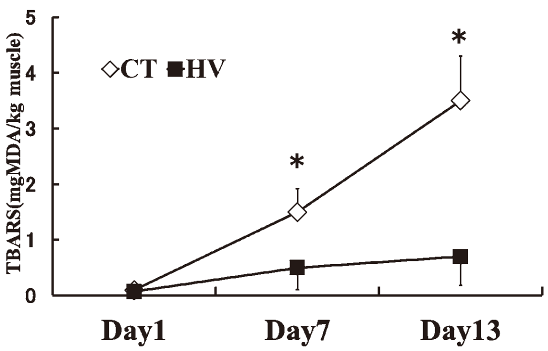

Fig. 3. Values of 2-Thiobarbituric acid reactive-substances (TBARS) in the longissimus muscle of *Wagyu* steers fed a control (CT) or high-vitamin (HV) diet. The data represent the means ± S.D. * Indicates a significant difference within each time point (P < 0.05). Based on data from Yamada et al. (2012).

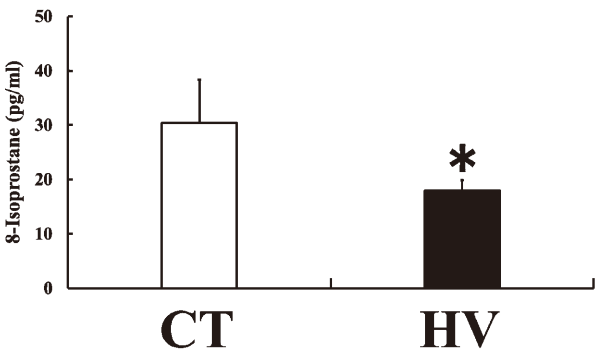

Fig. 4. Plasma levels of 8-isoprostane in *Wagyu* steers fed a control (CT) or high-vitamin (HV) diet. The data represent the means ± S.D. * P < 0.05. Based on data from Yamada et al. (2013).

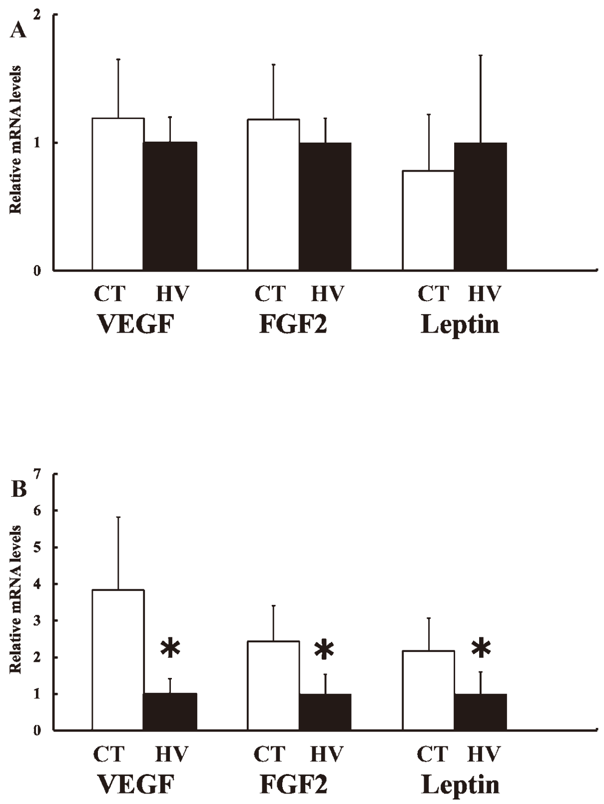

Fig. 5. Adipokine (VEGF, FGF-2 and leptin) gene expression in A) subcutaneous and B) mesenteric adipose tissue of *Wagyu* steers fed a control (CT) or high-vitamin (HV) diet. Ribosomal protein large P0 (RPLP0) mRNA was used as an internal control. The data represent the means ± S.D. * P < 0.05. Based on data from Yamada et al. (2013).

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