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Short communication

Effect of supplementation of purple pigment from anthocyanin-rich corn (*Zea mays* L.) on blood antioxidant activity and oxidation resistance in sheep

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ABSTRACT

Anthocyanins are pigments found widely in plants. They have been reported to possess antioxidant activity and an enhancing effect on superoxide dismutase (SOD) activity in monogastric animals. Thus, anthocyanins could also affect the status of the antioxidative defense system in ruminants. This study aimed to investigate the effect of the supplementation of purple pigment from anthocyanin-rich corn on blood antioxidant activities and oxidation resistance in sheep. Twelve sheep were used in a crossover design with two dietary treatments: a diet with purple corn pigment (pigment treatment) and one without (control). The pigment was added to the diets at an anthocyanin concentration of 0.5%. Blood and urine samples were collected to determine the markers of oxidative status. A portion of the plasma was used for an oxidation resistance test, in which plasma was incubated with an oxidizer at 37 °C for 0, 4 and 8 h, and then the degree of oxidation was measured. The pigment addition to the diet caused a significant increase in the SOD activity of the plasma, although the total antioxidant capacity and glutathione concentration in the plasma of both treatment groups were similar. In the oxidation resistance test, the oxidation in the plasma from the pigment treatment group was significantly suppressed compared to that in the control group at 4 and 8 h after the onset of the incubation. These findings indicate that the intake of purple pigment from anthocyanin-rich corn increases the SOD activity and the potential of the antioxidant activity of plasma in sheep.

1. Introduction

Oxidative stress arises when the production of free radicals surpasses the capacity of the natural antioxidative system in living organisms (Sies, 1991). The oxidative stress leads to peroxidative damage of cell membranes and macromolecules (Girotti, 1998), and could contribute to and/or get involved in the incidence of several disorders in cattle (Miller et al., 1993). It is reported that small ruminants such as sheep and goats can experience oxidative stress influenced by nutrition, season and physiological stage (Celi et al., 2010; Di Trana et al., 2006; Sgorlon et al., 2008). To cope with oxidative damage, the antioxidative defense is mostly managed by the actions of antioxidant substances that neutralize free radicals, and enzymes that catalyze radical- and peroxide-quenching reactions (Jacob, 1995).

Anthocyanins are pigments found widely in plants and are a type of polyphenol that exhibits antioxidant activity (Cevallos-Casals and Cisneros-Zevallos, 2003). Moreover, some anthocyanins have been reported to show enhancing effects on the mRNA expression and activity of superoxide dismutase (SOD), which is an important antioxidant enzyme in living organisms, in laboratory animals (Han et al., 2006; Sarić et al., 2009). Thus, these reports lead us to postulate that the use of anthocyanin in feed may affect the status of the antioxidative defense system in ruminant animals.

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In order to supply anthocyanin as an antioxidant substance to ruminant animals, the breeding of corn containing a great deal of anthocyanin for ruminant feed has been tried in Japan. An available anthocyanin-rich corn (*Zea mays* L.) has different nutrient composition and digestive characteristics in comparison with commercial common corn, although it contains a much higher amount of anthocyanin (Hosoda et al., in press). In a study that investigates the effect of antioxidant feeding on the oxidative status of animals, there is a possibility that no specific effect of antioxidant feeding is found when there is a difference in terms of nutrition between the dietary treatments, since a difference in nutrition could modulate oxidative status (Sgorlon et al., 2008).

To date, studies on the effect of the feeding of purple pigment from anthocyanin-rich corn on the status of antioxidative defense in sheep are not available. Our hypothesis is that anthocyanin feeding might improve the status of antioxidant substances and/or enzymes in sheep. To test this hypothesis, we measured the alteration of plasma antioxidant concentrations and activity and oxidation resistance in wethers fed a diet supplemented with purple corn pigment.

2. Materials and methods

2.1. Animals, experimental design and sampling

The animal experiment was conducted according to the Guide for the Care and Use of Experimental Animals (Animal Care Committee, National Institute of Livestock and Grassland Science). Twelve Suffolk wethers (57.4 ± 13.2 of BW) were kept individually in metabolic cages with free access to water. The experiment was performed as a replicated crossover design with 14-day periods, without an interval between the periods. The animals were provided a diet (Table 1) to meet the total digestible nutrients requirements for maintenance of the Japanese Feeding Standard for Sheep (AFFRCS, 1996), and were divided into one of two dietary treatment groups: a diet with purple corn pigment (pigment treatment) or one without (control). The pigment was added to the diets at an anthocyanin concentration of 0.5% diet on a dry matter (DM) basis. The pigment (Sunred NO.5 F) was purchased from San-Ei Gen F.F.I., Inc. (Osaka, Japan), and consisted of 78% pigment, 20% ethanol and 2% citric acid. Aqueous solution containing 20% ethanol and 2% citric acid was added to the diet in the control group to offer the same amount of ethanol and citric acid to the pigment treatment group. The final concentrations of ethanol and citric acid in the diets of both groups were 1.5 and 0.15%, respectively. Due to a lack of specific indications for the dose of anthocyanin in sheep, we employed an anthocyanin concentration that anthocyanin-rich corns should be able to contain in the future (personal communication) and also referred to a previous report involving mice (Tsuda et al., 2003). The diet was offered in equal amounts twice daily at 0900 h and 1700 h.

Table 1
Ingredient proportion and nutrient composition of the diet.

Item	
Ingredient proportion,% of DM	
Timothy hay	70.0
Flaked maize	16.0
Soybean meal	13.4
Dicalcium phosphate	0.3
Salt	0.3
Dry matter,%	89.1
Nutrient composition,% of DM	
Organic matter	94.4
Crude protein	12.7
Ether extract	1.9
Acid detergent fiber	30.9
Neutral detergent fiber	50.3
Crude ash	5.6

Feed refusals were recorded every morning, and the intakes by the animals were then calculated. On the last day of each period, blood samples were taken through an indwelling catheter in the jugular vein with heparinized tubes before and 4 h after morning feeding. The blood samples were centrifuged at 1200 × *g* for 15 min at 4 °C, and the supernatants were harvested. The samples were kept on ice or at −80 °C depending on the assays until the measurements were performed. Samples taken after morning feeding were used for the measurement of the oxidation resistance of plasma, and the samples taken both before and after morning feeding were used for the other assays. All urine was collected for 24 h prior to blood sampling, and urine samples were stored at −80 °C after the total weight of the urine was measured.

2.2. Chemical analysis

The DM of the fresh feed was determined by drying samples at 100 °C for 18 h. The feed samples were dried in an oven at 60 °C and ground through a 1-mm sieve. The air-dried sample was dried at 135 °C for 2 h to determine the DM. The crude protein, ether extract, and crude ash of the samples were analyzed according to AOAC Methods 976.05, 920.39, and 942.05, respectively (AOAC, 1990). The neutral detergent fiber and acid detergent fiber were determined by the method of Van Soest et al. (1991), and expressed free of ash.

Assays of the total antioxidant capacity (TAC, Test kit for Potential Anti Oxidant; Nikken SEIL, Shizuoka, Japan), which represents the non-enzymatic antioxidative systems (Straface et al., 2005), and total glutathione (GSH, Total Glutathione Quantification Kit; Dojindo Laboratories Kumamoto, Japan), as a non-enzymatic antioxidant, were performed using fresh plasma samples on ice within 24 h using commercial kits according to the manufacturer's instructions. A commercially available kit (SOD Assay Kit-WST; Dojindo Laboratories) was used to determine the SOD activity, as one of the endogenous enzymes, within a week after immediate extraction from the fresh plasma samples. Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a marker of oxidative stress was determined using a commercial ELISA kit (New 8-OHdG Check ELISA; Nikken SEIL) according to the manufacturer's instructions.

The oxidation resistance of plasma was measured by the method of the previous reports (Frei et al., 1988; Tsuda et al., 1998) with samples kept at −80 °C within 2 days from the collection. In brief, the plasma was incubated with oxidizer (2,2′-Azobis(2-methylpropionamidine) Dihydrochloride, final concentration: 25 mM) at 37 °C in a water bath. Portions of the mixture were withdrawn to a tube containing BHT (2,6-Di-t-