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Feed Intake, Nutrient Digestibility, Antioxidant Activity in Plasma, and Growth Performance of Male Dairy Cattle Fed Black Rice and Purple Corn Extracted Residue

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ABSTRACT

The study aimed to evaluate the impact of black rice and purple corn extracted residue (BPER) on feed intake, nutrient digestibility, growth performance, and antioxidant activity in male dairy cattle. The residue after extraction of anthocyanin from black rice and purple corn contains anthocyanin and phenolic acids. Several researchers found that anthocyanins and phenolic acids had antioxidant and antimicrobial functions in animals. Sixteen male dairy cattle (with average body weight of 160 ± 10.6 kg) were allotted in a completely randomized design (CRD) with 4 levels of black rice and purple corn extracted residue (BPER) at 0%, 2%, 4%, and 6% dry matter (DM) in total mixed ration (TMR). The TMR diets were fed *ad libitum* for 125 days. The daily DM intake, nutrient digestibility, growth performance, and plasma metabolites were measured. The results showed that BPER up to 6% had no effect on intakes of DM, organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF). Intake of ether extract (EE) increased with higher levels of BPER (p<0.05). The DM, OM, CP, NDF, and ADF digestibility were similar among treatments. The average daily gain (ADG) and feed per gain were similar among treatments. There was no effect of BPER on the concentrations of glucose, urea, total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), protein carbonyl, and antioxidant activity in the plasma. However, the malondialdehyde (MDA) concentrations in the plasma decreased (p<0.05) with the increased level of BPER, indicating that lower lipid oxidation compared to 0% BPER. It was concluded that BPER can be used up to 6% to reduce oxidative stress, without any negative effect on feed intake and nutrient digestibility.

***Keywords:** male dairy cattle; black rice and purple corn extracted residue; black rice bran residue; purple corn cob; digestibility; antioxidant*

INTRODUCTION

Crossbreeding of Holstein Friesian cattle is the most common breeding of dairy cattle in Thailand. The purpose of this crossbred is to improve the milk yield of *Bos Taurus* cattle having heat tolerance and resistance to tropical diseases. However, humidity, high levels of solar irradiance, and the air temperature of tropical locations negatively affect dairy cattle, causing heat stress in *Bos Taurus* cattle (Butt *et al.*, 2019). This heat stress condition generates free radicals, leading to oxidative stress (Akbarian *et al.*, 2016), which is the cause of chronic health problems in animals (Modzelewska-Kapituła *et al.*, 2018). Diets containing antioxidant compounds have antioxidative effects in decomposing and scavenging free radicals in cattle. As a result, there will be a balance between antioxidants and free radicals in the body (Ribeiro *et al.*, 2019).

Anthocyanins are the natural antioxidants having more antioxidant activities than vitamin E. Anthocyanins are found in plant pigments with a stable red-colored form under acidic condition and turn to blue-colored in the presence of a base condition (Fei *et al.*, 2021). The darker color could be explained by the high concentration of anthocyanin (Cömert *et al.*, 2020). Several researchers found that anthocyanins have antimicrobial effects that can act on the cytoplasmic membrane of bacteria and change its structure and function, leading to the loss of structural integrity of the cell membrane and they also play a role as antioxidants that their structures can be able to donate electrons to the free radicals with unpaired electrons and reduce agents in the pathway of electron-transfer reaction (Bendokas *et al.*, 2020; Prommachart *et al.*, 2020).

In Thailand, black rice (*Oryza sativa L.*) and purple corn (*Zea mays L.*) are important sources having the potential to be used as functional foods having antioxidants and food colorants because they are rich in anthocyanin and phenolic compounds (Kapcum *et al.*, 2018). Several studies reported the application of black

rice and purple corn. For example, supplementation of anthocyanin-rich purple corn extract orally improved oxidative stress status in rats (Kirisattayakul *et al.*, 2017), and oral supplementation of black-rice extract reduced oxidative stress status in humans and rats (Sangkitikomol *et al.*, 2010). In addition, the studies in the meat showed that black-rice extract retarded lipid oxidation and increased oxidative stability during storage of ground beef patties (Prommachart *et al.*, 2020), steak beef (Purba *et al.*, 2020), and pork patties (Park *et al.*, 2017). For these reasons, anthocyanin extracts from black rice and purple corn are produced industrially in response to consumer's demands. Consequently, after extract processing, there are a lot of agro-industrial residues from black rice and purple corn. On the contrary, residues from the extraction of anthocyanin still contain anthocyanin similar to those reported in grape pomace (Trikas *et al.*, 2016) and blackberry (Yamashita *et al.*, 2017).

Feeding agro-industrial residues to livestock reduce not only environmental problems caused by the accumulation of residues and decreases the carbon footprints of animal production (Gerber *et al.*, 2014) but also improve antioxidant status in animal (Tian *et al.*, 2019) due to the presence of bioactive compounds such as phytochemicals and vitamins.

Although the anthocyanin extracted from black rice and purple corn have antimicrobial and antioxidant functions, to the best of our knowledge, no published research has evaluated its by-product effect on improving antioxidant status to enhance the growth performance of cattle. Therefore, the objectives of this study were to evaluate the effect of black rice and purple corn extracted residue (BPER) on feed intake, nutrient digestibility, growth performance, and antioxidant activity in male dairy cattle.

MATERIALS AND METHODS

Animals, Treatments, and Experimental Design

This experiment was conducted at the Department of Animal Science, Khon Kaen University, Thailand. Regarding animal care, the guidelines recommended by the Animal Ethics Committee of Khon Kaen University were followed (U1-04090-2559). Sixteen males Holstein cattle (7-8 months) with an average body weight of 160 ± 10.6 kg were randomly assigned into one of four treatment groups (4 cattle per treatment) according to a completely randomized design (CRD). The cattle were raised 30 days for adaptation with *ad libitum* of TMR diet. The animals were kept indoors, housing with individual pens (2.5 m × 5 m) with concrete floors that were cleaned every morning. The water was available at all times. During the 125 days of the feeding trial, the BPER was added to TMR diets at the levels of 0, 2, 4, and 6%, respectively. All experimental cattle received experimental diets *ad libitum* twice a day. The TMR was formulated to provide 12% crude protein and 15 MJ/kg gross energy to meet the nutrient requirements of growing male dairy cattle according to NRC (2001). The ingredients and chemical composition of the experimental diets are shown in Table 1 and Table 2. The BPER was the residue consisted of 80% black-rice bran and 20% purple-corn cob (This proportion was taken by the manufacturing process) and obtained from Siam Miragro Company Limited, Khon Kean, Thailand. Digestibility was determined with external markers with chromic oxide (Cr_2O_3) according to the method described by de Souza *et al.* (2015). Briefly, a daily dose in paper capsule containing 10 g of Cr_2O_3 as a marker was orally administered in a single daily dose at the same time in the morning before feeding on day 110 to 120 of feeding trial.

Data Collection and Sample Analysis

Feed offered and feed refusals of the individual animals were recorded daily. The animals were weighed every 30 days before morning feeding for determination of weight gain. Feed efficiency was calculated by using feed intake and weight gain. Feed samples and fecal samples (grabbing sample technique) were collected on days 114 to 120 of the feeding trial. All samples were kept in plastic bags and stored at -20°C for subsequent analyses. Feed and fecal samples were dried at 60°C for 48 hours and ground prior to proximate analysis. At the end of the experiment, the samples were taken to the Nutrition Laboratory at the Department of Animal Science, Khon Kaen University and analyzed for DM (at 100ºC for 24 hours) according to AOAC (1995); CP was determined by total nitrogen determination using a 6.25 conversion factor according to Kjeldahl method of AOAC (1995); EE was determined by Soxhlet instrument using petroleum ether extract (AOAC, 1995); ash was determined (at 550ºC for 6 hours) according to AOAC (1995); NDF and ADF in samples were analyzed using amylase and sodium sulfite according to Van Soest *et al.* (1991) procedures; ADL (in 12 M H_2SO_4 for 3 hours) was determined according to Van Soest & Robertson (1980). The quantification of Cr_2O_3 in fecal samples was performed by the method described by Fenton & Fenton (1979). Briefly, 1 g of feed and feces samples were ashed at 600°C for 1 hour. After cooling, 15 mL of a digestion mixture was added to the samples and heated until a yellowness or redness. The digests were transferred to 250 mL volumetric flasks with distilled water and made up to volume. Stand overnight to allow suspended material to settle, then 10 mL of the diluted digest was centrifuged and measured at 370 nm using spectrophotometry to determine the Cr_2O_3 content compared with a standard curve. Organic matter (OM), cellulose, and hemicellulose were calculated using the following formulas, respectively: OM= 100 – ash; hemicellulose= NDF – ADF; cellulose= ADF – ADL. The GE levels of feed samples were measured using Isoperibol Bomb Calorimeter AC500 (Leco, Michigan, USA). Dry matter intake (DMI) was calculated at kg/day. Average daily feed intake (ADFI) was determined by dividing the total feed intake in terms of the days of trial and the number of each animal, expressed as a DM basis. The average daily gain (ADG) was calculated by dividing body weight gain based on the number of days.

The bioactive compounds of feed and BPER were determined by using samples (0.2 g) extracted twice

with 10 mL of acidified methanol dissolved in 1.0 N HCl (85:15 v/v) and incubated at room temperature for 2 hours with shaking. The extracts were centrifuged at 3,000 rpm at 4°C for 10 minutes, and the clear supernatants were collected into a volumetric flask. The total phenolic content in the feed and BPER were determined using Folin-Ciocalteu method (Ainsworth & Gillespie, 2007). Briefly, 200 μL of the extract was mixed with 600 μL Folin-Ciocalteu reagent (10%) and stood at room temperature for 5 minutes; 500 μL of Na_2CO_3 (700mM) solution was added to the mixture and incubated at room temperature for 2 hours. The absorbance was measured at 765 nm via a spectrophotometer. Gallic acid was used as the calibration standard, and the total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram of dry weight.

Total anthocyanin content was determined by the pH-differential method according to Lee *et al.* (2005). Briefly, two aliquots (0.5 mL) of the extracts were diluted (4.5 mL) with buffer reagents (0.025 M potassium chloride at $pH_{1.0}$ and 0.4 M sodium acetate at $pH_{4.5}$) and incubated in the dark for 20 minutes at room temperature. The absorbance was measured at 520 and 700 nm, respectively, using a UV1280-Vis Spectrophotometer (Shimadzu, Kyoto, Japan). The results were expressed in mg of cyanidin-3-O-glucoside equivalents per g dry weight using the equation:

Anthocyanins content (cyaniding-3-glucoside equivalents, mg/L) = $A \times MW \times DF \times 10^3/(\varepsilon \times 1)$

where A was $pH_{1.0}$ (A520 nm -A700 nm) – $pH_{4.5}$ (A520 nm -A700 nm), MW was molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF was dilution factor, 10^3 was factor for conversion from g to mg, ε was molar extinction coefficient of cyanidin-3-glucoside (26,900 $M^{-1}.cm^{-1}$), and 1 was the path length (cm).

Blood Sample Collection and Analysis

Blood samples for the plasma profile analyzed from each individual cow were collected before the morning meal at day 120 of the feeding trial by jugular venipuncture for 5 mL with a EDTA collection tube. The EDTA-plasma was separated by centrifugation (1500 rpm, 15 min), and stored at −80°C until analysis. The Roche cobas c501 automated analyzer (Roche Diagnostics Ltd, Rotkreuz, Switzerland) was used for quantification of total cholesterol (TC, enzymatic colorimetric CHOD-PAP method) and high-density lipoprotein cholesterol (HDL, enzymatic colorimetric PE-GPO-PAP method) concentrations. Plasma glucose (mg/mL), urea nitrogen (mg/dL), aspartate aminotransferase (AST, IU/L), and alanine aminotransferase (ALT, IU/L) were measured with enzymatic methods (Srinagarind Hospital, Faculty of Medicine of Khon Kaen University, Khon Kaen, Thailand).

The antioxidant capacity was determined by spectrophotometry in accordance with the method of Martinez *et al.* (2006), using a stable free radical a-diphenyl-b-picrylhydrazyl (DPPH). Briefly, 100 μL of plasma was mixed with 400 μL of methanol and centrifuged at 12,000 rpm at 4°C for 20 minutes to separate the proteins. The analysis was performed in microplates by adding 200 μL of 0.115 mM DPPH solution (dissolved in methanol) to 100 μL of clear supernatant and incubated in dark for 1 hour at room temperature. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer (SpectraMax® M3 Multi-Mode Microplate Reader, Molecular Devices, San Jose, CA, USA). The absorbance of DPPH with methanol (control sample) was used for a baseline measurement. The DPPH scavenging activity was calculated using the following equation:

DPPH scavenging activity (%) = $[(Ac - As) / Ac] \times 100$

where Ac is the absorbance of the control (DPPH solution with methanol) and As is the absorbance of the sample.

The lipid oxidation of plasma was determined with the malondialdehyde (MDA) using thiobarbituric acid reactive substances (TBARS), as described by Toaldo *et al.* (2015). Briefly, aliquots (200 μL) of plasma were acidified with 500 μL of 20% trichloroacetic acid (TCA) and 50 μL of 10 mM BHT. The 500 μL of Thiobarbituric acid (55mM) were added to the samples and incubated at 100°C in a water bath for 45 minutes. The samples were cooled on ice for 5 minutes, 1.5 mL of n-butanol were added and the mixtures were centrifuged at 10,000 rpm at 4°C for 10 minutes to obtain a clear supernatant. The absorbance was measured at 532 nm via a UV1280-Vis Spectrophotometer (Shimadzu, Kyoto, Japan) by running a blank that contained all reagents, except the plasma sample. The results were calculated as TBARS concentration expressed in $\mu molL^{-1}$ of MDA using the molar extinction coefficient of the pink TBA chromagen as 1.56×10^{-5}/M/cm.

The protein oxidation of plasma was assessed by assayed protein carbonyl using 2,4-dinitrophenylhydrazine (DNPH) base on the method of Patsoukis *et al.* (2004). Briefly, four aliquots of 100 μL of plasma were distributed in Eppendorf tubes. All aliquots were mixed with 1 mL of ice-cold TCA (10%) for 15 minutes in ice-baths to precipitate the proteins. The tube samples were centrifuged at 6,000 rpm for 10 minutes at 4°C to discard the clear supernatant. TCA (10%) was added (1 mL) to the pellets, and the procedures described above were repeated. After removing the supernatant, 500 μL of 10 mM DNPH (dissolved in 2.0 M HCl) were added to the pellets of two aliquots, and 500 μL of HCl (2.0 M) without DNPH were added to the pellet of two aliquots for blank. All samples were mixed and left in the dark for 1 hour, vortexed every 10 minutes to be derivative. Subsequently, 500 μL of TCA 20% was added to all samples, which were then vortexed, placed in ice baths for 15 minutes, and centrifuged at 8,000 rpm for 10 minutes in 4°C. The resulting supernatant was discarded. To remove excess DNPH, protein pellets were washed three times with 1 mL of ethanol: ethyl acetate (1:1, v/v), vortexed, and centrifuged at 8,000 rpm for 10 minutes in 4°C. After each wash, the supernatant was discarded. After the final wash, the samples were left under the hood for 20 minutes to remove the excess solvent, and the pellets were subsequently dissolved in 1.5 mL of 6.0 M guanidine hydrochloride (dissolved in 20 mM phosphate buffer, pH 6.5) and placed in the dark

for 30 minutes and vortexed every 10 minutes. Finally, the sample solution was centrifuged at 12,000 rpm for 10 minutes at 4°C to remove insoluble material. The carbonyl concentration in the samples was measured using spectrophotometry with absorbance at 370 nm and 280 nm using a UV1280-Vis Spectrophotometer (Shimadzu, Kyoto, Japan). The concentration of protein carbonyl was expressed as nmol carbonyl per mg protein (nmol/mg protein) and calculated using the following equation (Levine *et al*., 1994):

$$C_{hydrazone}/C_{protein} = \{A370/[\varepsilon_{hydrazone,370} \times (A_{280} - A_{370} \times 0.43)]\} \times 10^{6}\ [\text{nmol/mg protein}]$$

where $\varepsilon_{hydrazone}$,370 is 22,000 $M^{-1}cm^{-1}$ and 0.43 is $\varepsilon_{hydrazone,280}/\varepsilon_{hydrazone,370}$

Statistical Analysis

All data were statistically analyzed through analysis of variance (ANOVA) using the generalized linear model (GLM) procedure of the Statistical Analysis System (SAS, 1998). Results were presented as mean values with the standard error of the means. Orthogonal polynomials were used to evaluate for each parameter to determine linear and quadratic responses to supplementation of BPER, with the level of significance at p<0.05.

RESULTS

Chemical Composition

Feed ingredients and chemical compositions of treatment diets are presented in Table 1 and Table 2. The DM, organic matter (OM), crude protein (CP), ash, NDF, ADF, acid detergent lignin (ADL), hemicellulose, cellulose, and GE were similar in all treatments. The average total phenolic and anthocyanin content in BPER were 8.23 mg of gallic acid/g and 1.05 mg of cyanidin-3-O-glucoside equivalents per g dry weight, respec-

Table 1. Ingredients of experimental total mixed ration (TMR) diets

Ingredient composition (g/kg DM)	BPER, % of dietary DM			
	0	2	4	6
Cassava pulp	300	300	300	300
King Napier grass silage	243	243	243	243
Palm kernel meal	140	140	140	140
Cassava chip	110	110	110	110
Dried distillers corn grains with solubles	80	80	80	80
Defatted rice bran	100	80	60	40
Black rice and purple corn extracted residue	-	20	40	60
Salt	5	5	5	5
Sulfur	2	2	2	2
Dicalcium phosphate	5	5	5	5
Premix*	5	5	5	5
Urea	10	10	10	10
Total	1000	1000	1000	1000

Note: BPER= black rice and purple corn extracted residue; DM= dry matter; *= Each kilogram of premix contained the following: vitamin A= 3,000,000 IU; vitamin D= 600,000 IU; vitamin E= 9,000 IU; Fe= 50 g; Zn= 40 g; Mn= 40 g; Co= 0.1 g; Cu= 10 g; Se= 0.1 g; I= 0.5 g.

Table 2. Analyzed chemical composition and fatty acid profile of total mixed ration diets

Chemical composition (g/kg DM)	BPER, % of dietary DM				
	0	2	4	6	
Dry matter (DM, g/kg fresh weight)	387	392	395	385	938
Organic matter (OM)	937	938	939	939	955
Crude protein (CP)	124	128	123	125	137
Ether extract (EE)	23	27	33	36	128
Ash	62	62	61	61	102
Neutral detergent fiber (NDF)	432	437	424	423	325
Acid detergent fiber (ADF)	246	238	241	239	110
Acid detergent lignin (ADL)	53	55	52	54	36
Hemicellulose (NDF–ADF)	186	189	183	184	215
Cellulose (ADF–ADL)	193	188	192	187	74
Gross energy (MJ/kg DM)	15.23	15.13	15.29	15.2	17.29
Anthocyanins (mg/g DM)	ND	0.02	0.04	0.05	1.05
Total phenolic acid (mg gallic acid/g DM)	2.67	2.8	3.01	3.31	8.23

Note: BPER= black rice and purple corn extracted residue; ND= not detected; TMR= total mixed ration.

tively. The anthocyanin and total phenolic acid contents increased with a higher level of BPER in diet treatments, whereas the anthocyanin was not detected in the control sample (0% supplementation).

Feed Intake, Digestibility, and Growth Performances

The effects of BPER in the diet on feed intake are presented in Table 3. The results showed that BPER supplementation had no effect on total feed and nutrient intakes; however, fat intake increased with a higher level of BPER (p<0.05). The effects of BPER on the apparent digestibilities are presented in Table 4. There were no significant differences in apparent digestibilities of DM, OM, CP, NDF, and ADF. The growth performances of cattle are presented in Table 6. The final body weight, weight gain, average daily gain, and feed per gain were similar in all treatments.

Plasma Metabolite Profiles

The effects of BPER in the diet on plasma metabolite profiles are presented in Table 5. There were no significant effects of BPER supplementation on plasma concentrations of glucose, blood urea nitrogen, TC, HDL-cholesterol, AST, ALT, and antioxidant activity. However, MDA concentration was significantly different and linearly decreased with a higher level of BPER (p<0.05). Even though the higher values of BPER in the diets showed a slightly lower protein carbonyl, this difference was not statistically significant (p=0.07).

DISCUSSION

Bioactive Compounds of the Diets

The BPER in this experiment is the mixture of 80% black rice bran and 20% purple corn cob. This BPER contains 1.05 mg/g of anthocyanins and 8.25 mg gallic acid/g phenolic acid which are group of flavonoid compounds. Anthocyanins had high antioxidant activity (Cömert *et al.*, 2020) and could enhance antioxidant enzyme activity (Hosoda *et al.*, 2012a). Previous studies indicated that anthocyanin was not degraded during the fermentation process in the rumen (Tian *et al.*, 2018). In this experiment, BPER contained anthocyanin similar to those reported by Laokuldilok *et al.* (2011) at 1.13-2.56 mg/g of anthocyanin and 9.9% DM total phenolic acid for black rice bran. This concentration was higher than that reported by Hosoda *et al.* (2012b), who fed purple rice silage with anthocyanins concentration at 0.23 mg/g to sheep to improve oxidative status. It has been considered that BPER had bioactive ingredients and had potential antioxidant activity.

Effect of BPER on Intake and Digestibility of the Diets

Increasing BPER in the diet had no effect on DM intake and DM digestibility. The intake of CP, NDF, and ADF followed a similar pattern to DM intake. This might be because the BPER had no effect on cattle in terms of feed-sorting behavior and other factors which could be related to their behaviors. Several related fac-

Table 3. Voluntary feed intake and nutrient intake of male dairy cattle fed black rice and purple corn extracted residue in total mixed ration diets

Variables	BPER supplemented (%)				SEM	Trend	
	0	2	4	6		L	Q
Total feed intake (kg of DM)							
kg/d	6.65	6.84	7.23	7.08	0.44	0.45	0.72
%BW	2.66	2.73	2.80	2.68	0.06	0.68	0.20
g/kgBW0.75	105.32	108.16	111.84	107.58	2.75	0.44	0.25
Nutrient intake (kg DM/day)							
Organic matter	6.23	6.42	6.79	6.65	0.41	0.44	0.72
Crude protein	0.86	0.89	0.89	0.85	0.05	0.95	0.62
Ether extract	0.15	0.18	0.23	0.22	0.01	0.001	0.16
Neutral detergent fiber	2.87	2.99	3.06	2.99	0.24	0.64	0.65
Acid detergent fiber	1.63	1.62	1.74	1.69	0.1	0.59	0.85

Note: SEM= standard error of means; L= linear, Q= quadratic; BPER= black rice and purple corn extracted residue; TMR= total mixed ration; DM= dry matter.

Table 4. Apparent digestibility of male dairy cattle fed black rice and purple corn extracted residue in total mixed ration diets

Digestibility, %	BPER, % of dietary DM				SEM	Trend	
	0	2	4	6		L	Q
Dry matter	68.01	66.45	66.16	67.87	1.85	0.93	0.41
Organic matter	81.71	82.01	81.63	79.84	2.21	0.55	0.64
Crude protein	61.22	60.56	57.29	61.33	2.61	0.82	0.41
Neutral detergent fiber	49.46	48.61	47.63	48.03	2.74	0.69	0.83
Acid detergent fiber	40.68	38.57	38.02	39.79	3.12	0.83	0.56

Note: SEM= standard error of means; L= linear; Q= quadratic; BPER= black rice and purple corn extracted residue; TMR= total mixed ration; DM= dry matter.

Table 5. Plasma metabolite, liver enzymes, lipid oxidation, protein carbonyl, and antioxidant activities of male dairy cattle fed black rice and purple corn extracted residue in total mixed ration diets

Variables	ARE, % of dietary DM				SEM	Trend	
	0	2	4	6		L	Q
Glucose (mg/dL)	80.00	85.66	83.50	79.00	2.41	0.68	0.09
Urea nitrogen (mg/dL)	7.00	5.75	5.75	6.00	0.90	0.50	0.43
Total cholesterol (mg/dL)	99.00	103.50	99.07	92.25	8.25	0.56	0.50
HDL- cholesterol (mg/dL)	85.66	88.00	86.50	82.25	6.83	0.72	0.65
AST(IU/L)	52.25	48.67	48.25	56.00	4.48	0.60	0.25
ALT (IU/L)	20.50	19.50	20.25	21.33	1.74	0.70	0.57
MDA (umol/L)	0.47	0.38	0.28	0.30	0.05	0.02	0.26
Carbonyl protein (umol/L)	2.66	2.42	2.28	2.04	0.21	0.07	0.98
Antioxidant activity (%DPPH)	8.96	7.68	9.76	9.27	0.92	0.47	0.67

Note: SEM= standard error of means; L= linear; Q= quadratic; AST= aspartate aminotransferase; ALT= alanine aminotransferase; MDA= malondialdehyde; BPER= black rice and purple corn extracted residue; TMR= total mixed ration; DM= dry matter.

Table 6. Growth performance of male dairy cattle fed black rice and purple corn extracted residue in total mixed ration diets

Variables	BPER, % of dietary DM				SEM	Trend	
	0	2	4	6		L	Q
Initial body weight, kg	161.85	157.77	167.33	170.57	11.37	0.53	0.77
Final body weight, kg	351.37	351.80	355.33	365.07	22.80	0.69	0.85
Weight gain, kg	189.52	193.92	188.00	194.50	12.18	0.87	0.93
Average daily gain, kg	1.51	1.55	1.50	1.55	0.09	0.87	0.93
Feed per gain							
Dry matter intake	4.41	4.42	4.81	4.53	0.14	0.30	0.36
Organic matter intake	4.13	4.14	4.54	4.25	0.13	0.24	0.28
Crude protein intake	0.55	0.56	0.59	0.56	0.02	0.30	0.20

Note: SEM= standard error of means; L= linear; Q= quadratic; BPER= black rice and purple corn extracted residue; TMR= total mixed ration; DM= dry matter.

tors, including ruminal pH, volatile fatty acid profile, digesta flow rate, fiber digestion, and palatability, can affect cattle's feed intake (Hassan *et al.*, 2016).

Because flavonoid compounds are amphipathic molecules contained both hydrophilic and hydrophobic groups, they can interact with both the hydrophobic cores and the hydrophilic head groups of the lipid membranes, resulting in a high affinity with lipid compounds (Šturm & Poklar Ulrih, 2020). Therefore, the increase of flavonoid-compounds intake included anthocyanin and other phenolic compounds, will also result in the increase of ether extract intake (See Table 3). Similarly, Pereira *et al.* (2008) reported that steers fed *Brachiaria brizantha* silage (rich-flavonoid) had a higher intake of ether extract.

This result was similar to Oliveira *et al.* (2010), who reported that polyphenol-rich extract of dried pomegranate (5 to 10 g/day) had no impact on intake and DM digestibility, but it reduced the fat and protein digestions of calves. In addition, Hosoda *et al.* (2012a) reported that anthocyanin-rich corn silage had no effect on DM intake and total digestible nutrient intake of lactating dairy cows. These reported results might indicate that anthocyanin has no negative effect on the feed intake of ruminants.

However, the *in vitro* antimicrobial effect of anthocyanin had been reported (Zhao *et al.*, 2009). A high concentration of anthocyanins could have negative effects on microbial fermentation in the rumen of cattle, leading to the decreased nutrient digestibility. In this experiment, the BPER supplementation up to 6% in diets had no effect on the digestibility of DM, OM, NDF, and ADF. Therefore, the anthocyanin concentration in treatment diets might not be high enough to interfere with microbes in the rumen.

Plasma Metabolite

High intake of dietary energy could enhance mitochondrial free radical production, which causes oxidative stress (Fang *et al.*, 2002). Therefore, in this experiment, feeding TMR diets at *ad libitum* might increase the risk of oxidative stress. Plasma antioxidant activity is an indicator of the antioxidant status in the animal. The phenolic compounds were absorbed through the intestine of the digestive tract; a great variety of phenolic substances of plasma can enhance antioxidant capacity (Ishida *et al.*, 2015). Safari *et al.* (2018) reported that pomegranate by-product in the diets improved total antioxidant status in the plasma of dairy cows. However, in the present study, the plasma antioxidant capacity of cattle fed BPER diets was not improved. Similarly, Maciej *et al.* (2016) reported that oral flavonoid supplementation did not have any effect on the plasma anti-

oxidant capacity of newborn dairy cattle. Hosoda *et al.* (2012a) demonstrated that feeding anthocyanin-rich corn silage did not enhance plasma total antioxidant status in lactating dairy cows. Hosoda *et al.* (2012b) showed that supplementation of purple pigment from anthocyanin-rich corn did not enhance plasma total antioxidant capacity in sheep. When the level of antioxidants is low, their contributions to antioxidant capacity probably has little influence compared to endogenous antioxidative defense (Matthaiou *et al.,* 2014). The amount of BPER intake and/or period of experimentation could be limiting factors to improve antioxidant capacity. In addition, the small number of cattle replication (n=4) and endogenous antioxidant enzyme strong activity of young animal (Hatao *et al.,* 2006) might had influence on the not significant effect on the plasma antioxidant capacity of this study.

The MDA and protein carbonyl in plasma are byproducts derived from lipid and protein oxidations, respectively. This study demonstrated that the decreased MDA and protein carbonyl in plasma of cattle supplemented with more BPER in diets did not correlate with plasma total antioxidant.

The total antioxidant activity, which was not significantly different between treatments, showed the dominant effect of endogenous antioxidants. The decreasing levels of MDA and protein carbonyl showed the effect of antioxidant, anthocyanins, and phenolic acid (Ramos-Escudero *et al.,* 2012). The antioxidant effect of anthocyanin and phenolic did not have a significant effect on total antioxidant activity, presumably because the treatment level was not adequate. This is evidenced by a significant decrease in MDA and a tendency to decrease in protein carbonyl at the BPER level of 6%, but it has not had a significant effect on total antioxidant activity. This could be due to the activation of endogenous antioxidant enzymes affected by anthocyanins and phenolic acid on antioxidant status in the cell systems (Villasante *et al.,* 2016).

It has been reported that feeding natural flavonoid substances to animals significantly increased plasma superoxide dismutase (SOD) activity and decreased plasma MDA (Hosoda *et al.,* 2012a; Hosoda *et al.,* 2012b; Safari *et al*., 2018; Nurrofingah *et al*., 2020). Safari *et al.* (2018) demonstrated that phenolic acid from pomegranate by-products in diets of dairy cows had a positive effect on blood antioxidant by increasing blood antioxidant capacity and decreasing blood MDA concentration. Matsuba *et al.* (2019) who fed TMR purple corn silage (anthocyanins at 20-70 mg/kg) on lactating cows increased blood superoxide dismutase concentration. Similarly, the ingestion of conventional and organic red grape juice-rich polyphenols in humans decreased MDA values of plasma (Toaldo *et al.,* 2015).

CONCLUSION

The BPER supplementation up to 6% in the diet of male dairy cattle did not have any effect on feed intake, nutrient digestibility, and growth performance. There was no effect of BPER on metabolite, protein carbonyl, and antioxidant activity in plasma. However, the cattle fed BPER presented lower lipid oxidation in plasma. This indicates that BPER can be beneficial for health promotion by reducing oxidative stress in male dairy cattle.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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