Research Note: Effects of supplementing cranberry and blueberry pomaces on meat quality and antioxidative capacity in broilers

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ABSTRACT Cranberry and blueberry pomaces are rich in antimicrobial and antioxidant compounds. They have been identified as potential antibiotic alternatives in animal feed, but their antioxidative capacity for maintaining or improving the meat quality in broilers is not well documented. This study was to determine whether cranberry and wild blueberry pomaces in diets could positively influence the broiler meat quality. A total of 3,150 broilers were randomly allotted to 10 dietary treatments with bacitracin methylene disalicylate, wild cranberry pomace (**CRP**) (0.5 and 1% of the basal diet), and wild blueberry pomace (**BLP**) (0.5 and 1% of the basal diet) alone or in combination with a mixture of feed enzymes. The results showed that supplementation with the CRP or BLP did not affect meat lightness and yellowness, while the deeper red meat (higher a* values) was observed in the birds receiving the diet containing 0.5% BLP against those in CRP treatments ($P = 0.015$). In addition, inclusion of CRP or BLP in the diet did not change meat texture and proximate composition (moisture, protein, fat, ash) irrespective of pomace concentrations. Although there were no obvious effects of CRP or BLP supplementation on meat antioxidant capacity and the incidence of myopathies ($P > 0.05$), the upward trend of antioxidant capacity and less severity of woody breast were observed in birds fed with 0.5% CRP. Furthermore, supplementation of 0.5 or 1.0% CRP without the enzyme resulted in higher mRNA levers of *Nrf*, *Gpx2*, and *HO-1* ($P < 0.05$). Taken together, 0.5% CRP supplementation without the enzyme could potentially maintain meat quality and attenuate the severity of woody breast.

Key words: broiler, fruit pomace, meat quality, antioxidative capacity, woody breast

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INTRODUCTION

With the global increase in antibiotic resistance and restrictions in antibiotics use, there is an urgent need to identify antibiotic alternatives to maintain or improve livestock and poultry health and performance. Pomace is a by-product of the fruit-processing industry, and it has been suggested as a promising antimicrobial alternative in feed (Ross et al., 2017). In recent years, the global poultry industry has been suffering from the emerging breast myopathy (Kuttappan et al., 2016). They include 1) the white-striping (**WS**) defect exhibiting white striations parallel to the muscle fibers on the ventral surface of breast fillets; 2) the woody breast (**WB**) condition (often associated with the WS defect) in which affected muscles are visually hard, bulging out, and exhibiting pale color. The breast myopathy can result in poultry meat of lower quality, leading to additional processing costs, downgrading products, and the potential condemnation of meat (Kuttappan et al., 2016). It is conservatively estimated that the incidence of the WB costs over $200 million USD annually in the United States alone (Kuttappan et al., 2016). The oxidative stress in

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the muscle was associated with the occurrence of breast myopathy (Cai et al., 2018). The pomace is rich in plant polyphenol, which can enhance the antioxidant activity. However, whether the pomace could exert antioxidant functions to alleviate the occurrence of muscle oxidative stress and consequently reduce the incidence and severity of breast myopathy is not well documented.

Both blueberry and cranberry are economically important crops in Canada (Ross et al., 2017). Pomaces are mass-produced as by-products from fruit processing, and they can be used as an alternative feedstuff to potentially replace antibiotics. Das et al. (2020) have reported that the supplementation of cranberry pomace (**CRP**) or blueberry pomace (**BLP**) significantly increased the body weight during the starting and growing phases and reduced the incidence of necrotic enteritis in commercial broilers. However, whether supplementation of CRP or BLP could positively influence the meat quality and mitigate the incidence of the breast myopathy was not clear. Furthermore, feeding pomace with feed enzymes may lead to further release of nutrients and functional components that can influence metabolism and increase release of components from carbohydrate matrix (Kiarie et al., 2013). Thus, this study was to determine whether supplementing different concentrations of CRP or BLP with or without an enzyme complex in diets could positively influence the broiler meat quality, antioxidative capacity, and breast myopathy.

MATERIALS AND METHODS

Cranberry and Blueberry Pomaces

Organic cranberry and low-bush blueberry pomace were used to generate either freeze-died CRP or low-bush BLP. Crude protein, ash, fat, soluble dietary fiber, insoluble dietary fiber, and moisture analyses were performed according to the Association of Official Analytical Chemists International procedures. Total phenolics, tartaric esters, flavonols, and anthocyanins were assessed with Glories method, and antioxidant activity were assessed with the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid and ferric reducing antioxidant power methods as previously described (Ross et al., 2017).

Management and Treatment

A total of 3,150, 1-day-old Cobb 500 chicks were raised in a floor pen barn. The birds were randomly assigned to 10 dietary treatments with bacitracin methylene disalicylate (**BMD**), wild CRP (0.5 and 1% of the basal diet), and wild BLP (0.5 and 1% of the basal diet) alone or in combination with a mixture of enzymes (7 pens/treatment, 45 chicks/pen) over a 5-wk experimental period. Chicks and feed were obtained from Sollio Agriculture s.e.c., Montreal, Quebec, Canada, and Agri-Marche Inc., Saint-Isidore, Quebec, Canada, respectively. The enzyme mixture was included at 500 g/ton and contained cellulase (minimum 2,800 CMC units/g), mannanase (minimum 400 MAN units/g), galactanase (minimum 50 GAL units/g), xylanase (minimum 1,000 XYL units/g), glucanase (minimum 600 GLU units/g), amylase (minimum 2,500 FAA units/g), protease (minimum 200 HUT units/g; Canadian Bio-System, Calgary, Alberta, Canada). The barn temperature was initially set at 34°C and then gradually reduced by 2°C each week to reach 24°C at 35 d of age. Chicks were exposed to light for 24 h on the first day, 23 h on the second, 18 h on the third, and 16 h thereafter. Starter (day 1–14), grower (14–28), and finisher (28–35) diets were formulated with wheat, barley, and corn as the principal cereals and soya meal as protein concentrates to meet the Cobb nutritional requirements. The detailed compositions for these diets were described in the article by Das et al. (2020).

All experimental procedures performed in this study were approved by the Animal Care Committee of the Centre de recherche en sciences animales de Deschambault (protocol #1920-AV-397, CRSAD, Deschambault, Quebec, Canada) according to guidelines described by the Canadian Council on Animal Care (CCAC, 2009).

Sampling

On day 35, one bird per pen (7/treatment) with a weight close to the group mean was randomly selected and euthanized. Immediately after euthanasia, left breast meats were sampled, and about 30 g of meat was separated for freeze-drying, 2 g meat and 1 g meat were separated and stored at −80°C for the assay for antioxidant activities and real-time quantitative PCR (**RT-qRCR**), respectively, and the rest was stored on the ice for detection of meat color and texture.

Measurement of Meat Color and Texture

The lightness (L*), redness (a*), and yellowness (b*) values were determined with a spectrometer (CM-3500d; Konica Minolta, Tokyo, Japan) in triplicate on the ventral indefective (no discolorations or petechial bruises) surface of the fresh breast meat. The shear force and shear energy values were measured using a TA.XT plus texture analyzer (Stable Micro Systems Ltd., Surrey, UK), with a 5-kg load cell and a Meullenet-Owens Razor Shear Blade.

Measurement of Proximate Composition

The moisture content of chicken was measured by using a freeze-dryer. About 30 g of fresh meat was freeze-dried at −140°C for 72 h until a constant weight was reached and stored in a desiccator. Then the samples were minced in an electric grinder. Protein content was determined with 50 mg of freeze-dried samples by using the Dumas Nitrogen Analyzer (NDA 701; Velp Scientifica, Monza and Brianza, Italy). The total nitrogen level was converted to protein content using a conversion factor of 6.25. The ash content of a sample was the residue

left after ashing in a muffle furnace at about 600°C for 6 h till the residue became white. The intramuscular fat in 5.0 g of ground samples was extracted with 60 ml of diethyl ether using a Soxhlet Extractor (SER 148; Velp Scientifica).

Scoring of the WS and WB

The left pectoralis *major filets* were palpated and scored for WS as described in the study by Kuttappan et al. (2016) and for WB as described in the study by Tijare et al. (2016).

The WS was categorized as 0 = no distinct white lines (normal); 1 = small white lines, generally < 1-mm thick, but apparently visible on the fillet surface (moderate); 2 = large white lines (1- to 2-mm thick) very visible on the fillet surface (severe); 3 = thick white bands (>2 mm thickness) covering almost the entire surface of fillet (extreme).

The WB were categorized as 0 = fillets that are flexible throughout (normal); 1 = fillets that are hard mainly in the cranial region but flexible otherwise (moderate); 2 = fillets that are hard throughout but flexible in the mid-to-caudal region (severe); 3 = fillets that are extremely hard and rigid throughout from cranial region to caudal tip (extreme).

Assay for Antioxidant Activity

For antioxidant analyses, about 0.5 g of samples were homogenized in ice-cold phosphate buffer (1 × , pH 7) and centrifuged at 12,000 rpm at 4°C for 10 min. The supernatant was collected for total antioxidant capacity (**TAC**) analysis using QuantiChrom Antioxidant Assay Kit (DTAC-100; BioAssay Systems, Hayward, CA).

RNA Extraction and RT-qPCR

Total RNA was extracted from the breast tissue using Trizol (Applied Biosystems, Waltham, MA). The RNA was resuspended in RNase-free water, and the concentration and purity were measured using a NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE). RNA (1 µg) was mixed with RNase Inhibitor (Applied Biosystems) for reverse transcription in accordance with the kit protocol (Applied Biosystems). The process included an initial step at 25°C for 10 min, followed by incubation at 37°C for 120 min and 85°C for 5 min; cDNA was stored at −80°C. The antioxidant-related genes detected included nuclear factor E2-related factor 2 (***Nrf2***), glutamate cysteine ligase catalytic subunit (***Gclc***), glutathione peroxidase 2 (***Gpx2***), thioredoxin (***Txn***), and heme oxygenase-1(***HO-1***). The RT-qPCR assay was carried out on a CFX Connect Real-Time PCR Detection System (Bio-Rad, Irvine, CA) with samples containing 5 µL of SYBR Green Master Mix, 0.2 µL forward and reverse primer, 1 µL cDNA template, and 3.6 µL RNA-free water. Reaction conditions included one cycle at 98°C for 2 min and 40 cycles of 98°C for 10 s and 60°C for 30 s. Experiments detecting all genes were performed in triplicate, and expression levels were assessed relative to chicken β-actin as an internal standard. Mean ΔCt values of control birds for BMD supplementation with the mix enzyme were used as calibrators. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analyses

Data were analyzed as a 2 × 5 (enzyme × supplementation treatment) factorial arrangement of treatments by 2-way ANOVA. The model included the main effects of adding enzyme and antibiotic substitute and their interaction using a general linear model procedure of the SPSS statistical software (SPSS, ver. 19.0, Chicago, IL). Differences in means among treatments were tested using the least significant difference method, and significant differences were set at $P \leq 0.05$.

RESULTS

Composition of the Pomaces Used in This Study

Freeze-dried BLP had more crude protein and soluble dietary fiber contents than the CRP. However, CRP showed the highest level of insoluble dietary fiber. As expected, BLP showed the highest total phenolics (1,342.40 vs. 733.05 mg of galic acid equivalent/100 g dry wt); tartaric esters (463.41 vs. 259.55 mg caffeic acid/100 g dry wt), flavonols (445.46 vs. 353.21 mg quercetin/100 g dry wt), anthocyanins (604.11 vs. 92.53 mg cyanidin 3-glucoside/100 g dry w), and antioxidant activities (10,760.11 vs. 7,269.79 µmol Trol Eq/100 g dw) compared with the CRP.

Effects of Cranberry or Blueberry Pomace Supplementations on Color, Texture, and Proximate Composition of Breast Meat

The effects of replacing bacitracin with CRP or BLP on color, texture, and proximate composition of breast meat are shown in Table 1. Supplementation with CRP or BLP in growing broilers did not compromise meat lightness, yellowness, and tenderness compared with the diet with BMD supplement irrespective of the tested enzyme mixture. However, inclusion of BLP0.5 in the diet resulted in darker meat (higher a* values) than that in the CRP supplementation ($P = 0.015$), but not different from BMD. In addition, supplementing the enzyme complex did not affect the meat color and texture.

The proximate composition (moisture, protein, fat, ash) of meat from broilers was analyzed. Diet supplementation with CRP1 or BLP1 did not change the proximate composition (moisture, protein, and fat) irrespective of pomace concentrations or addition of the enzyme complex, but BLP tended to increase the ash content ($P = 0.08$) in comparison with the BMD

Table 1. Effects of cranberry and blueberry pomace supplementations on meat color, texture, proximate composition, myopathy, and antioxidant activity in broilers.[1]

	Meat color			Meat texture		Proximate composition				Myopathy		Antioxidant
Item	L* (light)	a* (red)	b* (yellowness)	SF(N)	SE (N* mm)	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Score of WS	Score of WB	TAC (μM Trolox)
With enzyme												
BMD	48.72	1.48	7.79	32.61	17.27	72.85	21.96	2.13	1.34	2.14	1.86	357.09
CRP0.5	48.57	1.54	7.23	33.43	16.92	72.04	22.94	1.77	1.40	2.14	1.57	387.91
CRP1.0	50.16	1.09	7.97	30.10	16.12	74.01	21.07	2.11	1.38	2.46	2.23	379.48
BLP0.5	48.19	2.03	8.20	30.80	16.94	71.67	22.95	1.99	1.50	2.29	1.71	334.69
BLP1.0	48.97	1.17	7.23	33.17	17.25	72.75	21.97	1.65	1.47	2.14	1.86	350.73
Without enzyme												
BMD	48.28	1.76	8.28	27.41	14.58	69.37	22.34	2.06	1.41	2.29	2.14	342.56
CRP0.5	49.32	1.35	7.90	28.22	15.03	72.96	22.02	2.00	1.38	2.29	1.71	373.12
CRP1.0	48.40	1.14	7.50	29.01	15.17	72.30	22.88	2.08	1.43	2.43	1.86	384.87
BLP0.5	48.76	1.97	8.29	30.18	15.67	72.41	22.47	2.13	1.45	2.14	1.86	354.87
BLP1.0	49.78	1.66	8.91	28.40	14.82	72.03	22.40	1.80	1.52	2.14	1.57	359.57
SEM	3.38	0.08	0.15	0.95	0.55	0.39	0.36	0.09	0.02	0.09	0.09	7.47
Main factors												
Enzyme												
With	48.90	1.46	7.68	32.02	16.90	72.66	22.18	1.93	1.42	2.18	1.85	361.98
Without	48.91	1.58	8.18	28.60	15.04	72.45	22.44	2.33	1.44	2.26	1.83	363.00
Supplementation treatment												
BMD	48.50	1.62^{a,b}	8.03	30.01	15.93	71.11	22.14	2.10	1.37	2.21	2.00	349.83
CRP0.5	48.90	1.45^{b}	7.56	30.83	15.98	72.46	22.52	1.87	1.39	2.21	1.64	380.52
CRP1.0	49.28	1.12^{b}	7.73	29.55	15.64	73.15	21.98	2.10	1.40	2.45	2.10	382.18
BLP0.5	48.47	2.00^{a}	8.25	30.52	16.36	72.04	22.73	2.07	1.48	2.21	1.79	344.78
BLP1.0	49.37	1.41^{b}	8.07	30.78	16.04	72.39	22.18	1.71	1.49	2.14	1.71	355.15
P value												
Enzyme	0.992	0.474	0.100	0.091	0.117	0.651	0.565	0.677	0.464	0.908	0.926	0.947
Supplementation treatment	0.704	0.015	0.600	0.993	0.998	0.550	0.821	0.708	0.083	0.848	0.570	0.391
Enzyme*supplementation treatment	0.443	0.666	0.230	0.894	0.988	0.291	0.296	0.986	0.722	0.988	0.729	0.938

Abbreviations: BMD, bacitracin methylene disalicylate; BLP, blueberry pomace; CRP, cranberry pomace; SF, shear force; TAC, total antioxidant capacity; WB, woody breast; WS, white-striping.
Means with different superscript letters indicate significant difference ($P < 0.05$).
[1]Data represent means and SEM of 7 replicates/treatment.

supplement, while the corresponding values for birds fed with the pomace (BLP or CRP) and BMD were 1.39–1.49 and 1.37%, respectively.

Effects of Cranberry or Blueberry Pomace Supplementation on the Incidence of the WS and WB and Antioxidant Activity in Broilers

The scores of WS and WB in broilers were evaluated, and the TAC of the treated birds is also presented in Table 1. No differences were observed for the main effects of the enzyme treatment or pomace supplementation. However, the average WB score for the broilers receiving BMD was 2.0, indicating severe myopathy according to the WB scoring system, while the corresponding values for birds supplemented with CRP 0.5, BLP 0.5, and BLP 1.0 were 1.64, 1.79, and 1.71, respectively, classified as relatively mild symptoms.

As for the TAC, there were no significant effects of the enzyme treatment and supplementation treatment (pomace or BMD). However, there was numerically higher TAC from the cranberry supplementation than from the BMD supplementation (380.52 μM Trolox for CRP 0.5, 382.18 μM Trolox for CRP 1.0, and 349.83 μM Trolox for BMD, respectively).

Effects of Cranberry or Blueberry Pomace Supplementations on Antioxidant-Related Gene Expression in Broilers

Expression of 5 antioxidant-related genes (*Nrf2*, *Gclc*, *Gpx2*, *Txn*, and *HO-1*) of the treated birds is shown in Figure 1. Expression of *Nrf2* and *Gpx2* mRNAs was significantly higher in the birds receiving either CRP 0.5 or CRP1.0 without enzyme supplementation than in those fed BMD. Also, a significantly higher *HO-1* mRNA lever was observed for the CRP1.0 treatment.

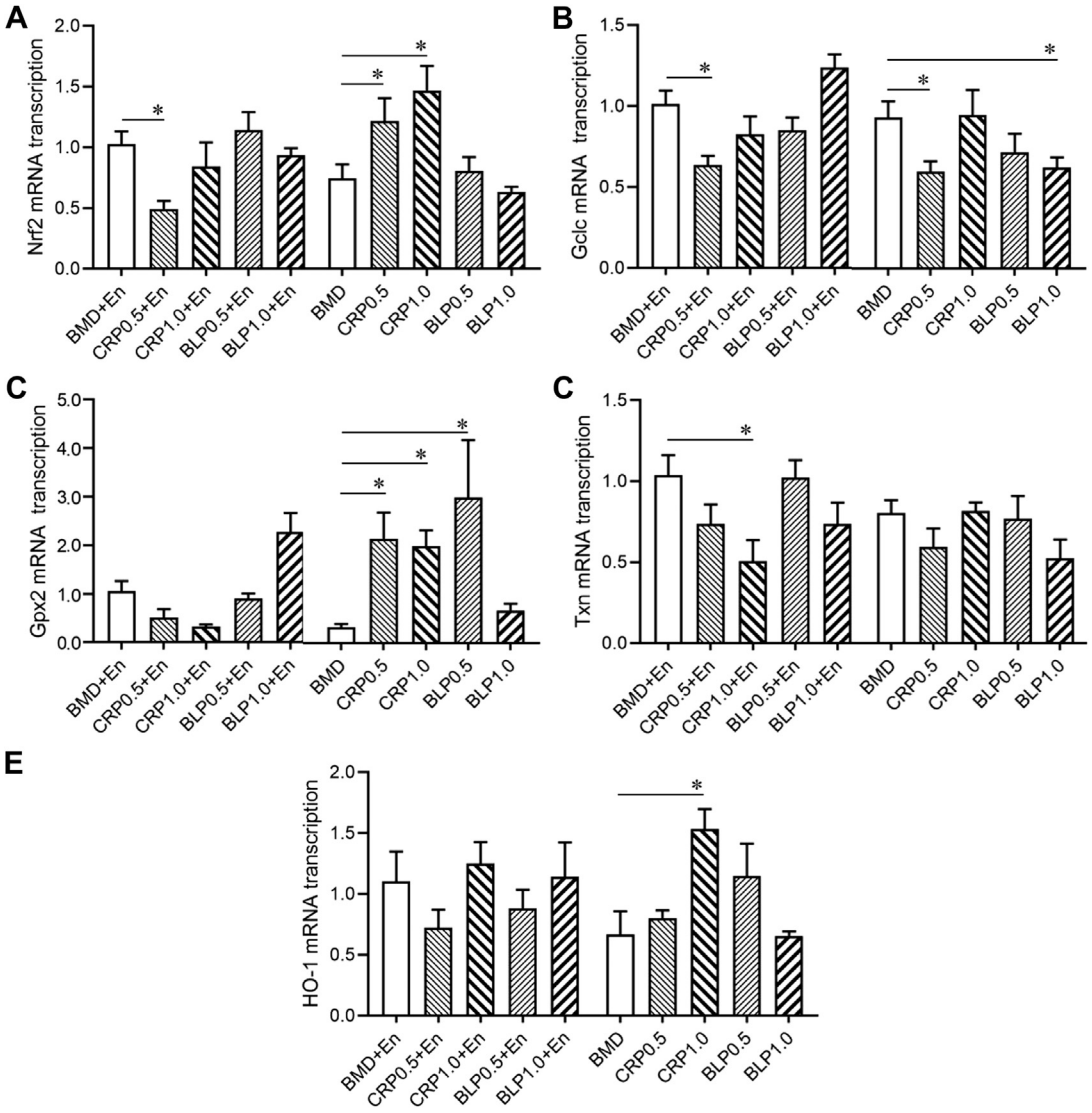

Figure 1. Effects of cranberry and blueberry pomace supplementations on expression of 5 antioxidant-related genes, including *Nrf2* (A), *Gclc* (B), *Gpx2* (C), *Txn* (D), and *HO-1* (E). "+ En" meant supplementation with mixture of enzymes. Gene expression was determined using RT-qPCR and is represented relative to β-actin. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Vertical bars represent the mean ± SD (n = 3). Significant differences relative to controls are indicated with asterisks ($P < 0.05$).

However, CRP with the enzyme supplementation resulted in lower mRNA expression of *Nrf2*, *Gclc*, and *Txn*. In addition, the BLP supplementation hardly affected the antioxidant-related genes' expression except for *Gclc* and *Gpx2*.

DISCUSSION

Berry fruit pomace represents a potentially useful resource for poultry diets because of their nutritive values (carbohydrates, proteins, minerals, vitamins, and so on) in addition to the potential health-promoting benefits derived from polyphenolic compounds (Das et al., 2020). In the present study, meat color, texture, proximate composition, and the antioxidant-related properties of broilers receiving different concentrations of CRP or BLP in feed were measured. Meat color is one of the most important appearance traits because consumers use it as an indication of quality and freshness. In the present study, feed supplementation with the CRP or BLP did not affect meat lightness and yellowness compared with BMD regardless of the tested enzyme mixture in feed. Similarly, Kasapidou et al. (2013) showed that the addition of the grape pomace to the diet did not have a significant impact on the meat L* and b* values compared with the control. However, inclusion of BLP0.5 in the diet affected redness and resulted in deeper red meat than CRP supplementation. Thus, BLP may promote more myoglobin deposition in meat than does CRP. In addition, feed supplementation with CRP or BLP did not impair meat tenderness compared with BMD, similar to the finding from a previous study (Kasapidou et al., 2013), in which dietary grape pomace did not influence the eating quality of the chicken compared with the control (Kasapidou et al., 2013). Moreover, feed supplementation with CRP or BLP up to 1% diet did not affect the proximate composition (moisture, protein, fat, ash) compared with BMD.

The myopathy severity and antioxidant capacity were not statistically different in CRP- and BLP-fed broilers compared with those in broilers treated with BMD in the present study. Nevertheless, the WB symptoms tended to be lower in broilers in CRP (0.5%) and BLP (0.5 and 1.0%) treatments than in those in the BMD treatment. Also, birds receiving CRP at 0.5% showed numerically higher antioxidant capacities than those receiving BMD (380.52 μM Trolox for CRP 0.5 vs. 349.83 μM Trolox for BMD). These results suggest that supplementing 0.5% CRP has a potential to alleviate the WB symptoms. Despite a higher antioxidant activity of BLP than CRP, meat from BLP-fed bird showed a lower antioxidant activity than the meat from CRP-fed birds. These data indicated that the ability of a pomace to influence the meat antioxidant status could be driven by a complex mechanism independent to the level the in vitro measured antioxidant activity. WB is mainly caused by oxidative stress injury (Cai et al., 2018). Considering that the BDM supplementation could enhance antioxidant enzyme activities in broilers compared with antibiotic-free negative birds, our results indicated that CRP and BLP had beneficial antioxidant effects similar to those of the BDM treatment.

The effect of the pomace on expression of 5 antioxidant-related genes was further investigated. However, there was no consistent effect of CRP or BLP supplementation on the expression of *Nrf2*-mediated response genes, including *Gclc*, *Gpx2*, *HO-1*, and *Txn*. These antioxidant genes are involved in glutathione production and regeneration, reactive oxygen species elimination, phase II detoxifying enzymes metabolism, and *Txn*-based antioxidant system (Tonelli et al., 2018). Feed supplementation with CRP at 0.5 or 1.0% without enzyme uniformly resulted in high mRNA levels of *Nrf2*, *Gpx2*, and *HO-1*. These results suggested that CRP was likely to exert function of antioxidant activity via reactive oxygen species elimination and xenobiotic detoxification. Whether the change of antioxidant-related genes at the mRNA level would translate into more proteins and increase the antioxidant capacity is expected but not confirmed because of the lack of corresponding antibodies.

The enzyme complex in our experiment was used in the diets to break down fiber and complex carbohydrates for enhancing plant-polyphenolic bioavailability. The mixture of enzymes included cellulase, mannanase, galactanase, xylanase, glucanase, amylase, and protease, but did not contain pectinase and tannase. We did not observe any beneficial effects on antioxidant activities by the supplementation of the enzyme complex. More work is needed to find suitable enzyme complexes to increase bioactive compounds from pomaces.

It is concluded that the feed supplementation with CRP did not affect the meat color, texture, proximate composition, nor the antioxidant-related properties in comparison to those treated with BMD. However, the upward trend of antioxidant capacity and reduced severity of WB were observed in chickens fed 0.5% CRP compared with those fed BMD.

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DISCLOSURES

The authors declare that they have no competing interests.

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