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Running title: Functional value of fruit pomaces in poultry

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Abstract

Nutritive and functional value of fruit pomaces in poultry is unexplored. We determined apparent metabolizable energy (AME) and plasma metabolites in broiler chickens fed diets containing apple (APL), low bush wild blueberry (LBP), cranberry (CRP) and grape (GRP) pomaces without or with multi-enzyme supplement (ENZ). A total of 360 one-d old Ross 708 male chicks were placed in 72 cages; 5 birds/cage, were reared to d 13 and transitioned to either cornstarch-soy protein isolate basal diet or basal with 30% of either pomace without or with ENZ. Excreta samples were collected from d 17 to 20 and one bird/cage bled for plasma metabolites on d 21. Apple pomace showed a higher AME ($P=0.008$) than other pomaces, however, ENZ had no effect on AME. The AME was 3,250, 2,613, 2,394 and 3,008 kcal/kg DM for APL, LBP, CRP, and GRP, respectively. There was pomace and ENZ interaction on plasma alkaline phosphatase ($P = 0.04$) and APL increased cholesterol levels ($P < 0.01$). In conclusion, ENZ had no impact on energy increment in pomaces, but the AME values this study has established are nonetheless valuable for accurate poultry feed formulation. Plasma metabolites suggested pomace components are amenable to supplemental ENZ.

Keywords: Broiler chickens, feed enzymes, fruit pomaces, apparent metabolizable energy, plasma metabolites

Introduction

Pomaces are residues of fruit processing, composed of the fleshy pericarp, peels, seeds, stems and other parts specific to fruit being processed (Ross et al. 2017). Pomaces may be produced after mechanical juice extraction whilst others are produced by use of chemical means. The chemical methods render the pomaces to be technically inorganically produced, as is with other feed ingredients such as solvent treated versus mechanically extruded soybean meal (CFIA 2019; Leung et al. 2020).

About 90% of apples (**APL**) in Canada are mainly produced in British Columbia, Ontario, and Quebec, with Nova Scotia trailing the three provinces. Various studies reported that dried APL pomace contained ~ 43.6 % dietary fibers (Sato et al. 2010), mono and disaccharides such as sucrose, arabinose, galactose and fructose (Gabriel et al. 2013), triterpenoids (Almeida-Trasviña et al. 2014), polyphenolic compounds (Vrhovsek et al. 2004), crude protein of up to 7.1% and macro- and micro-elements such as sodium, potassium, calcium, phosphorus, magnesium, iron, manganese, zinc, and copper (Pieszka et al. 2015). Grape (**GRP**) is cultivated in Canada mainly for the wine industry and produced extensively in the Okanagan valley (Canada 2021). Characterization efforts by Makris *et al* (2013) demonstrated a wide array of polyphenolic phytochemicals such as catechins, stilbenes, saponins, flavanols, anthocyanins, phenylpropanoids and derivatives of *p*-coumaric and benzoic acids. Studies by Silva Soares (2018) demonstrated the anthelmintic, ovicidal and antilarval properties of saponins and tannins found in GRP, up to a certain level, beyond which the tannins would then have an anti-nutritive effect due to the strong binding complexes it forms with proteins and macromolecules in the body.

Wild-blueberry, also called low bush-blueberry, (*Vaccinium angustifolium*) and organic cranberries (*Vaccinium macrocarpon*) are grown in Canada principally in the Atlantic, Quebec

and British Columbia provinces (Sandra Behm 2020; Turner 2009). Bioactive characterization of organic cranberry pomace (**CRP**) and blueberry pomace (**LBP**) by Ross et al (2017) revealed a wide assortment of chemical consistent such as carbohydrates including soluble and insoluble fibers, protein, minerals, phenolics such as flavonols, anthocyanins and tannins. Even though many tannins are considered to be anti-nutritive factors in terms of reducing iron absorption, protein digestibility and even growth, its potential as an anti-carcinogen, antibacterial, and antioxidant as well as an immune-modulator agent among other advantages have been demonstrated (Ashok et al. 2012).

Apple (Aghili et al. 2019), GRP (Gungor et al. 2021; Hosseini-Vashan et al. 2020), CRP (Islam et al. 2020) and LBP (Islam et al. 2019) pomaces have been studied for their use in chicken production to different ends, but with low adoption rates commercially. Considering the variation in chemical composition in pomaces, complex carbohydrates and fibers, the digestibility and availability of nutrients of these pomaces in the chicken gut by the endogenous enzymes are unknown. Research has shown that exogenous enzymes in poultry feed breakdown cell wall matrices and other anti-nutritive components of feed to release starch, protein, and fats, making them available for absorption in the gastrointestinal tract (Gallardo et al. 2017; Karimi et al. 2013). Gallardo et al (2017) further indicated that the use of multi-carbohydrase enzyme with canola meal could improve nutrient and energy utilization and fiber (non-starch polysaccharides (NSP) solubilization for effective hindgut fermentation in broiler chicks. This breakdown is associated with degradation of the anti-nutritive factors such as NSP and phytic acid in feed (Kiarie et al. 2016) and leads to the release of starch, fats, proteins, minerals and energy release according to Slominski (2011). The use of a cocktail of enzymes instead of single pure enzymes could be beneficial to improve availability of nutrients encased in structurally complex ingredients such as pomaces (Ravindran 2013; Kithama et al., 2021).

Little is known about the potential of fruit pomaces in broiler production, specifically metabolizable energy content. Such knowledge is needed to accurately formulate feed with fruit pomaces for poultry. Moreover, even though beneficial effects of pomace consumption on blood metabolites has been sporadically demonstrated in broiler models (Das et al. 2020; Islam et al. 2020; Islam et al. 2019), the benefits or impact on physiological and biochemical status of broilers has not been extensively studied. Therefore, it is paramount to evaluate the pomace effects on the plasma biochemical profile of chickens. The general objective of this study was to evaluate nutritive and functional value of APL, GRP, CRP, and LBP fruit pomaces fed without or with a multi-enzyme supplement (**ENZ**) in broiler chickens. The specific objectives were to evaluate the apparent metabolizable energy (**AME**) of pomaces and plasma biochemical profile in broiler chickens. It was hypothesized that feeding pomace with feed enzymes will lead to further release of nutrients and functional components.

Materials and Methods

Pomaces and experimental diets

Frozen wet organic CRP and LBP pomaces were obtained from Fruit D'Or (Villeroy, QC, Canada). Apple and GRP pomaces were procured from processors (Bennett's Apples & Cider, and Niagara College Teaching Winery/The Canadian Food and Wine Institute, Niagara Falls, ON). The pomaces were freeze-dried and then ground to pass through a 2 mm mesh screen using a cutting mill (SM 2000 Retsch, Haan, Germany) and stored at -20°C until use. A basal semi-purified corn starch and soybean protein isolate (CP, 87.3%) diet (Table 1) was formulated to contain 3,000 kcal/kg AME and 20% crude protein to meet or exceed minerals and vitamins specifications for a growing broiler (Aviagen 2016). The pomace diets were created by mixing

basal diet and respective pomace at 7:3 (wt/wt) ratio (Kiarie et al. 2014; Ravindran et al. 2014; Woyengo et al. 2010). Each of pomace diet was then supplemented with ENZ containing galactanase, protease, β -mannanase, β -glucanase, xylanase, α -amylase and cellulase activities at 50, 200, 400, 600, 7,000, 2,500, and, 2,800 U/g of product, respectively (Superzyme EO®, Canadian Bio-systems Inc., Calgary, AB Canada). Thus, the eight pomace diets and the control basal diet gave a total of nine dietary treatments. The basal diet was fed to facilitate calculation of AME by difference method (Kiarie et al. 2014; Ravindran et al. 2014; Woyengo et al. 2010). The diets contained 0.25% titanium dioxide indigestible marker and were prepared in mash form.

Birds and Housing

The present study was conducted at the Arkell Poultry Research Station and approved by the University of Guelph Animal Ethics Committee (Animal User Protocol no. 3521) according to the Canadian Council of Animal Care guidelines (CCAC 2009). A total of 360-d old Ross 708 male chicks were purchased from a local hatchery (Maple Leaf Foods, New Hamburg, ON, Canada), weighed individually, assigned to 72 identical metabolic cages (5 birds/cage). Cages (24" $\times$ 20" $\times$ 18") were placed in an environmentally controlled room. The room temperature was set at 32°C on d 0 and this was gradually decreased to 27°C by d 21. The lighting program was 24 h of light (20+ LUX) from day 0 to 3, followed by 20 h of light (10-15 LUX) for subsequent days to day 21. Birds had free access to feed and water through the nipple drinkers and troughs respectively.

Experimental Procedures

Broilers were fed a commercial starter diet for the first 13 d of life for adaptation. The commercial starter was corn and soybean meal based with concentrations of crude protein, crude fat, crude fiber, Ca, and P being 22.8, 4.7, 2.8, 0.97 and 0.62%, respectively (Floradale Feed Mill Ltd., Floradale, ON, Canada). On d 14, the nine diets allocated in a completely

randomized design (8 replicate cages per diet) based on cage body weight (BW). Birds had free access to feed and water throughout the study. Feed intake (**FI**) as well as mortality were monitored between d 14 and 21.

Sample collection

Fresh excreta samples were collected from each cage consecutively from d 17 to 20 and stored at -20°C (Woyengo et al. 2010). On d 21, one bird per cage was bled for plasma in heparin coated tubes, samples placed on ice and transferred to the laboratory. The blood was spun at 4,000 g, 4°C for 15 min to separate out the plasma layer, which was then stored at -20°C.

Sample Processing: Plasma, excreta and exogenous enzyme analyses

Plasma biochemical profile including protein, enzymes, metabolites, and minerals were analyzed by photometric method as described by Greenacre et al (2008) at the Animal Health Laboratory (University of Guelph, Guelph, ON). Excreta samples were pooled, and oven dried for four days at 60°C. The pomaces, diets and excreta samples were ground in a coffee grinder (Proctor Silex Fresh Grind™ Coffee Grinder) and mixed thoroughly for analyses. All samples were analyzed for the following: dry matter (**DM**), nitrogen (**N**), crude fat (**CF**), neutral detergent fiber (**NDF**), minerals, and gross energy (**GE**). Pomaces were further analyzed analysed for polyphenolics and non-starch polysaccharide mono sugars and diets for amino acids. Dry matter was determined according to method of AOAC (2005; method 925.09) (AOAC 2005) and Nitrogen by Leco Elemental Analyzer 828, Michigan, USA. The NDF and ADF were analyzed according to methods described by Van Soest *et al.*, (Van Soest 1994) using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY), and ether extraction was done using hexane according to AOAC methods (2005; method 982.30) (AOAC 2005).

Gross energy was determined using a bomb calorimeter (IKA Calorimeter System C 5000; IKA Works, Wilmington, NC). Mineral content was analysed according to AOAC (2005; method 968.08), ash by AOAC (2005; method 942.05) starch by AOAC (2005; method 996.11) and ethanol soluble carbohydrates according to Maness (2010). Titanium content in diets and excreta was measured on a UV spectrophotometer (Myers et al. 2004).

For amino acids (AA) analyses, samples were prepared by acid hydrolysis according AOAC (2005, method 982.30). Briefly, about 100 mg of each sample was digested in 4 mL of 6 N HCl for 24 h at 110°C, followed by neutralization with 4 mL of 25% (wt/vol) NaOH and cooled to room temperature. The mixture was then equalized to 50 mL volume with sodium citrate buffer (pH 2.2) and analyzed using Ultra performance liquid chromatography (UPLC, Waters Corporation, Millford, CA, USA). Samples for analysis of sulfur containing AA (Met and Cys) were subjected to performic acid oxidation prior to acid hydrolysis. Tryptophan was not determined. Total phenolics, tartaric esters, anthocyanins and flavonols in pomaces were measured using Glories method as described by Harrison et al. (2013) and Ross et al. (2017). Non-starch polysaccharide mono sugars were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedure described by Englyst and Cummings (1984; 1988) with modifications (Slominski et al. 2006). Xylanase activity in pomace diets was assayed using Xylazyme AX tablets (Megazyme International Ltd., Bray, Ireland). One unit of xylanase was defined as the quantity of the enzyme that liberated 1 μmol of xylose equivalent per min.

Calculation and statistical analyses

The apparent retention of components (dry matter, crude protein, crude fat, NDF and gross energy) were calculated according to Adeola (2000) using the following equation:

$$\text{Apparent Retention (\%)} = 100 - [100 \times (M_{\text{feed}} \times C_{\text{excreta}} / M_{\text{excreta}} \times C_{\text{feed}})]$$

Where:

M_{feed} and $M_{excreta}$ are the concentrations of index (TiO_2) in feed and excreta respectively, whilst $C_{excreta}$ and C_{feed} represent the concentrations of the components (energy) in faeces and feed respectively.

The apparent metabolizable energy (AME) in APL, CRP, LBP and GRP was calculated using the following formula by Ravindran *et al.* (2014):

$$AME_{APL, CRP, LBP \text{ or } GRP} \text{ (kcal/kg)} = \frac{AME \text{ of APL, CRP, LBP, or GRP diets} - (AME \text{ basal diet} \times 0.70)}{0.30}$$

The cage was the experimental unit for all parameters measured. Data were normal (Gaussian) and analyzed as a completely randomized design using the General Linear Mixed Model (GLMM) procedure of SAS 9.4 (SAS 2016), that would account for variation in the fixed effects, interactions and assumed correlated errors. The model had pomace, ENZ, and associated interactions as fixed factors. Multiple comparisons using Tukey test was used to separate pomace and interaction LSmeans and independent t-test for ENZ whenever the F-value was significant. A *P*-value of 0.05 or less was used to declare significance.

Results

Chemical composition of pomaces and experimental diets

The analyzed chemical composition of the pomaces is presented in Table 2. The CRP and LBP showed comparable DM of ~ 98%, whereas APL and GRP DM were 92 and 92.2%, respectively. Crude protein content was higher in LBP (11.6%), followed by CRP (7.4%), while APL and GRP showed a CP content of ~4% for both. The gross energy for the four pomaces were higher for LBP (5,388 kcal kg⁻¹) and CRP (5,534 kcal kg⁻¹), compared to APL

(4,188 kcal kg⁻¹) and GRP (4,865 kcal kg⁻¹). The crude fat content was higher in LBP (10.3%) and CRP (8.7%) than in the APL and GRP (Table 2). The highest crude protein concentration among the four pomaces were observed in the LBP (11.8%) and CRP (7.6%). The profile of AA revealed a low content of Lys, Met and Cys in all pomaces. The most abundant AAs, specifically in CRP, LBP and GRP included Phe, Val, Ala, Pro, Ser and Tyr. Among the four studied pomaces, CRP and GRP showed the highest content of Arg (Table 2). Carbohydrate composition differed greatly among the four pomaces, with GRP starch content being the highest (22.0%) compared to 2% for CRP and LBP, and 8.8% for APL. The NDF fraction on the other hand stood at 72.6% and 75.0% for LBP and CRP, respectively, but GRP and APL showed NDF values of 9.6% and 34.4%, respectively. The phenolics content presented in Table 2 indicate marked differences between the pomaces, with CRP and LBP pomace having the highest concentrations of total phenolics, tartaric esters, flavonols and anthocyanins than APL and GRP.

As presented in Table 3, mixing pomaces with the basal diet increased the levels of crude fat while the crude protein content did not markedly change. Contents of AA such as Arg, Ile, Pro, Phe, and Glu were higher in CRP diet than in the other diets. Xylanase activity was determined to confirm accuracy of inclusion of ENZ and feed mixing. The analyzed xylanase activity in pomace diets without ENZ 25, 55, 119, and 29 XU/g for APL, CRP, LBP, and GRP, respectively. Corresponding xylanase activity pomace diets treated with ENZ was 8,348, 7561, 7,097 and 9,000 XU/kg for APL, CRP, LBP, and GRP, respectively.

Apparent Metabolizable Energy (AME) and apparent retention of nutrients

The birds readily consumed experimental diets and there was no mortality throughout the experimental period. The average feed intake for the 7 experimental period was 249, 146, 280,

298 and 251 g bird⁻¹ for the basal, APL, LBP, CRP and GRP diets, respectively. Data in Table 4 shows that there was no interaction between ENZ and pomace for apparent retention of DM ($P=0.369$), N ($P=0.068$), CF ($P=0.784$), GE ($P=0.878$) and AME ($P=0.852$) but there was for NDF ($P=0.045$). The interaction was such that, the apparent retention of NDF for APL fed with ENZ was lower than for non-ENZ group. The main effects of pomace were significant for apparent retention of DM ($P=0.002$), N ($P<0.001$), NDF ($P<0.001$), GE ($P<0.001$) and AME ($P=0.008$). The APL showed higher apparent retention of GE (76.2%) than CRP (64.6%), LBP (66.2%), and GRP pomace (70.4%). Similarly, the highest AME (3,349 kcal kg⁻¹) was observed with APL compared to CRP (2,394 kcal kg⁻¹), LBP (2,612 kcal kg⁻¹), and GRP (3,008 kcal kg⁻¹). Supplemental ENZ had no effect on AME ($P=0.109$).

Plasma metabolites

There was interaction ($P=0.04$) between pomace and ENZ on plasma concentration of alkaline phosphatase and urea (Table 5). Specifically, feeding GRP with ENZ resulted in a higher Alkaline phosphatase than APL with ENZ or CRP without ENZ ($P\leq 0.04$). With respect to urea, birds fed GRP without ENZ had lower plasma concentration of urea than birds fed GRP with ENZ (Table 5). Main effect of pomace was noted ($P<0.01$) for plasma concentration of bile acid and cholesterol. The ($P < 0.01$) lowest concentration of cholesterol and bile acids was found in birds fed CRP, LBP and GRP diets compared to the birds fed with APL pomace. There was an interaction ($P=0.008$) between pomace and ENZ on plasma concentration of phosphorous and sodium (Table 5). Plasma phosphorus in birds fed APL with ENZ was higher than for birds fed LBP and GRP without ENZ. Whereas birds fed GRP without ENZ exhibited higher plasma sodium than birds fed LBP and CRP with ENZ. Birds fed APL, LBP and GRP had higher ($P<0.05$) concentration of plasma CO₂ than birds fed CRP, however, APL birds had higher ($P<0.05$) concentration. Birds fed APL and GRP had higher plasma calcium than birds

fed LBP and CRP. Birds fed CRP had lower plasma potassium compared with birds fed other pomaces (Table 5).

Discussion

The type, source and nutrient composition of feed ingredients incorporated in broiler diets have profound effects on the overall growth and development of the birds. The four tested pomaces in the present study showed differences in their chemical components: significantly lower crude protein contents in APL and GRP than in LBP and CRP, higher starch in APL and GRP than in LBP and CRP and higher crude fat and gross energy in CRP and LBP. The amino acid profile of the pomaces did not impact the dietary crude protein levels, and as such the contribution of the amino acids is negligible. The chemical profile APL used in the present study differed from that of Aghili (2019) which reported significantly high levels of crude fat, and fiber; this can be attributed to the variety used and processing methods. Similar crude fat values with present study were obtained by Taranu (2018) for GRP, even though their NDF and ADF were much higher in tested pomace. A higher NDF and ADF content was observed in the CRP and LBP-diets compared to the APL and GRP diets, relating directly to uniqueness of pomaces. Fiber plays an important role in poultry nutrition, with implications on feed intake, gut physiology, gut motility, nutrient absorption and gut microbiota (Tejeda and Kim 2021; Kiarie et al. 2014).

The polyphenolic compounds showed high variability, with LBP having the highest concentrations of total phenolics, tartaric esters, flavonols and anthocyanins. Phenolic compounds have been extensively studied and shown to exhibit beneficial biological effects including antimicrobial, anti-inflammatory and antioxidant properties (Harrison et al. 2013). The differences in fruit cultivars, species, processing methods *et cetera* will produce pomaces that vary considerably in their chemical compositions which could influence their in vivo

activities in poultry. These are areas which should be explored in future studies to standardize the products and optimize them for broiler production.

Even though APL, GRP, LBP and CRP pomaces have been used sporadically in horses, rabbits, chicken and swine diets (Brenes et al. 2016; Islam et al. 2020; Islam et al. 2019; Nicodemus et al. 2007; Taranu et al. 2018), there is still a big interest in their use due to emerging feed technologies that will help in optimizing their benefits by taking advantage on their wide range of nutrients and polyphenolic compounds having antimicrobial and antioxidant properties (Islam et al. 2020). Fiber matrix of pomaces encapsulates poly- and oligosaccharides, and polyphenols that when released by fiber degrading enzymes would be of great benefit to broiler chickens (Kithama et al., 2021). In the present study the semi-purified diets were used to minimize confounding effects of other dietary components on AME determination. The experimental period and the diets used in this study were not optimized for growth performance evaluation. Apple pomace birds had less feed intake relative to birds fed basal and other pomaces. Studies by Aghili (2019) showed that feeding APL pomace, with and without enzyme, decreased feed intake and reduced performance in broilers. Similar studies with APL pomace also showed reduced feed intake, postulated to be because of high fiber content (Heidarisafer et al. 2016).

Energy is a property of nutrients derived from the catabolism of carbohydrates, lipids and protein in feed, with various feed ingredients, having different energy levels that could be used in diets to meet the energy requirements for broiler chickens (Kocher et al. 2003). The available energy content of ingredients for poultry, which determines the feed intake, could be measured through different methods including measurement of apparent metabolizable energy (AME) (Hill and Anderson 1958). The tested fruit pomaces in the present study varied gross energy retention and subsequently AME. Higher AME contents in CRP and LBP compared to APL and GRP

might be attributable to the higher content of crude fat, neutral- and acid- detergent fiber and crude protein, even though APL and GRP had higher starch content. Although there are not many studies documenting the AME of fruit pomaces in poultry. One study showed that the GRP pomace in their poultry diets had an AME of 2,433 kcal/kg (Hosseini-Vashan et al. 2020). It has been reported that GRP pomace at 0.5%, 7.5% and 1% inclusion level had an ileal digestibility of gross energy of 81.73%, 79.42% and 77.08%, respectively in broiler chickens (Aditya et al. 2018). However, feeding GRP to horses (Kolláthová et al. 2020) showed improved digestibility of nutrients due to its polyphenolics aiding nutrient uptake, as was also observed in ruminants (Makkar 2003). Experiments with similar enzyme admixtures used in the present study showed higher AME values with corn and soybean meal based diets than control diets (Kocher et al. 2003). In our study, no significant effects of the ENZ used were observed on the energy and digestibility values of studied pomaces. Perhaps suggesting that the ENZ used in the present study was not optimized for pomaces.

Dietary fiber in poultry research is seen from a two dimensional angle; one as a functional component for normal physiological functions of the gut, and two as an anti-nutrient (Tejeda and Kim 2021), and variability in assessing the digestibility of fiber in broiler diets is highly influenced by the fiber source, type, and diet formulation (Sanchez et al., 2022). Neutral detergent fiber is composed of hemicellulose, cellulose and lignin component of plants, and data from the present study showed NDF retention was variable among the four pomaces. Among the pomaces, APL diets with or without enzyme had negative retention values for NDF indicating increased flow of dietary and endogenous NDF in the excreta. The mechanism for this phenomenon is unknown but lower feed intake in birds fed APL could be a reason. In addition, the physicochemical characteristic of APL fiber and ensuing gastrointestinal effects could also

have contributed. For, example broiler chickens (Thanabalan et al. 2020) fed flaxseed exhibited reduced retention of NDF which was associated with mucilage.

The avian plasma biochemical profile is an important tool used by veterinarians in the diagnosis of diseases and conditions of commercial, pet, and wild species of birds. Fruit pomaces can be a significant source of components entrapped in the fiber matrix. These includes flavonoids, phenolic acids, and stilbenoids (Islam et al. 2020), which exert non-specific effects on living organisms and regulate the activity of enzymes and cell receptors. For example, polyphenol-rich extracts from CRP fruits displayed potential in increasing energy efficiency, insulin sensitivity, and in decreasing triglyceride and cholesterol contents. In the present study avian biochemistry was evaluated to characterize the physiological impact of APL, LBP, CRP and GRP pomaces at an inclusion rate of 30% with or without an enzymes-mixture in feed on various blood plasma metabolite levels. Measurement and assessment of chicken blood parameters does not have standards and protocols, therefore different equipment for measurement will give varied results compared to another equipment or methodology (Brugere-Picoux 2015).

The total protein content provides some information regarding general status; however, more clinically useful data are indicated by plasma albumin and globulin ratio (Brugere-Picoux (2015); Harr (2002). The total protein or albumin and globulin were not affected by dietary pomaces however, a trend was observed for enzyme to increase total protein and globulin and subsequently reduce the albumin to globulin ratio. These proteins are primarily synthesized in the liver and play important roles in the fat metabolism (Greenacre et al. 2008; Harr 2002). The primary factors affecting albumin and globulin synthesis include protein and amino acid nutrition, colloidal osmotic pressure, the action of certain hormones, and disease status (Harr 2002). Thus, the tendency for higher circulating plasma globulin observed in birds fed enzyme

could be linked to increase release of nutrients in the gut. Circulating plasma enzymes reflects damage to an organ or tissue particularly the liver and kidney (Rozenboim et al. 2016). Alkaline phosphatase (ALP) is found in bile ducts, bone, liver, intestine, placenta, and tumors (Sharma et al. 2014). Elevations of the blood alkaline phosphatase level occur with hepatobiliary disease but also during healing fractures, vitamin D deficiency, bone disease and malignancy (Brugere-Picoux 2015; Sharma et al. 2014). Enzyme treated GRP pomace had a significantly higher plasma ALP compared to the rest of the enzyme treated and non-enzyme treated pomaces. Increase in plasma alkaline phosphatase has also been noted in pigs fed diets supplemented with phytase enzyme linked with increased bone mineralization (Kiarie et al. 2022). Additionally, there was non-significant differences in ALP levels between the enzyme treated and non-enzyme treated pomaces. High cholesterol levels in blood are indicative of obesity with liver steatosis, high lipids in diet or fasting (Brugere-Picoux 2015). With consideration of these underlying factors, the low feed intake of the birds on APL supplemented diet may be the attributable factor to the high cholesterol even though more studies are warranted regarding impact of APL diets on lipid metabolism.

Plasma sodium, potassium and phosphorus levels were within normal range compared to the baseline, and there was an interaction between pomace and enzyme for sodium and phosphorus levels) (Brugere-Picoux (2015); Harr (2002). Decrease in plasma potassium could be an indication of diuretic inefficiencies in birds, while an increase in its levels would indicate a case of kidney disease or dehydration (Brugere-Picoux 2015). Similar kidney disease symptoms would be observed in the case of increase phosphorus levels in plasma, while decreased quantities would lead to bone disorders (Brugere-Picoux 2015; Sharma et al. 2014). Calcium concentration in plasma also plays a major role in bone modeling, kidney conditions and metabolism of vitamin D. In the present study, APL pomace-fed birds showed the highest

level of plasma calcium compared to the other four pomaces. These observations in conjunction with ALP responses suggested feeding pomace with enzyme influenced mineral metabolism perhaps linked to increased release of bioactives related to mineral utilization in broilers. A study on horses fed GRP pomace showed similar effects on plasma mineral levels (Kolláthová et al. 2020).

In conclusion, present results showed that considering AME, the four fruit pomaces tested can be used in poultry rations. These high inclusion rates (30%) in the present study did not show adverse effects based on plasma metabolites, however, we hypothesize that the high fiber fraction may have contributed to the low feed intake of the birds. It should be noted that lower LBP and CRP inclusion rates (1 - 2%) have been applied in broiler chicken feeding programs for a longer period (30 days) without notable detrimental effects (Das et al 2020). Based on high fiber and other phytochemical contents, cranberry and blueberry pomaces could be explored for efficient use in poultry. In the present study, the diets were semi-purified, and so future studies should aim at including the pomaces into standard corn-soybean based or wheat-based diets and their assessment at lower inclusion levels to optimize their benefits and animal performance.

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Competing Interests

The authors declare there are no competing interests.

Author Contributions' Statement

EK, KR and MSD contributed resources and study design and data analysis. MK conducted investigation and wrote the paper. MSD and EK reviewed and edited the manuscript and provided overall supervision.

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Data Statement

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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Table 1. Composition of basal diet, as fed basis

Ingredient	%
Corn starch	57.4
Soybean protein isolate	23.0
Soy oil	6.61
Sucrose	5.00
Cellulose	3.00
Limestone	1.67
Mono calcium phosphate	1.43
Vitamin and trace minerals premix ¹	1.00
Salt	0.26
Titanium dioxide	0.25
Potassium carbonate	0.24
Sodium bicarbonate	0.10
Magnesium oxide	0.09
Calculated provisions	
AME, kcal/kg	3,150
Crude protein, %	20.0
Standardized ileal digestible Lys, %	1.11
Standardized ileal digestible Met % + Cys, %	0.53
Standardized ileal digestible Thr, %	0.63
Calcium, %	0.90
Available P, %	0.43
Na, %	0.23
Cl, %	0.22

¹Provided per kilogram of diet: vitamin A, 8,800.0 IU; vitamin D₃, 3,300.0 IU; vitamin E, 40.0 IU; vitamin B₁₂, 12.0 mg; vitamin K₃, 3.3 mg; niacin, 50.0 mg; choline, 1,200.0 mg; folic acid, 1.0 mg; biotin, 0.22 mg; pyridoxine, 3.3 mg; thiamine, 4.0 mg; calcium pantothenic acid, 15.0 mg; riboflavin, 8.0 mg; manganese, 70.0 mg; zinc, 70.0 mg; iron, 60.0 mg; iodine, 1.0 mg; copper, 10 mg; and selenium, 0.3 mg.

Table 2. Analyzed chemical composition of pomaces, as fed basis

Item	Apple	Blueberry	Cranberry	Grape
Dry matter, %	92.1	98.6	98.3	92.2
Carbohydrates, %				
Starch	8.75	1.97	1.97	22.04
Ethanol soluble carbohydrates	33.58	6.76	4.90	55.32
Neutral detergent fiber	34.42	72.59	75.04	9.63
Acid detergent fiber	12.76	46.50	59.49	6.19
Mono sugars				
Arabinose	2.92	1.11	4.56	0.39
Xylose	1.46	1.06	3.19	3.17
Mannose	0.41	0.47	0.80	0.46
Galactose	1.56	1.39	1.93	0.52
Glucose	8.50	9.60	17.63	5.85
Uronic acid	12.77	13.55	16.04	6.02
Total non-starch polysaccharides	27.63	27.19	44.15	16.40
Polyphenols				
Total phenolics – Glories (mg GAE 100 g ⁻¹ dry wt)	377.5	1342.4	733.1	661.0
Tartaric esters – Glories (mg caffeic acid 100 g ⁻¹ dry wt)	99.5	463.4	259.6	121.5
Flavonols - Glories (mg quercetin 100 g ⁻¹ dry wt)	109.5	445.5	353.2	82.5
Anthocyanins - Glories (mg Cyan 3-glu 100 g ⁻¹ dry wt)	8.50	604.1	92.5	21.5

Table 3. Analyzed composition of experimental diets, as fed basis

Item	Control	Apple	Blueberry	Cranberry	Grape
Dry matter, %	92.6	94.5	94.5	94.7	92.0
Gross energy, kcal kg ⁻¹	3,939	3,881	4,428	4,460	4,077
Crude fat, %	1.94	2.21	5.86	9.33	4.56
Crude protein, %	16.2	16.4	15.3	18.0	17.8
Amino acids, %					
Arg	1.85	1.85	0.04	0.02	2.06
His	0.44	0.44	0.23	0.20	0.05
Ile	0.61	0.61	0.82	0.84	1.01
Leu	1.34	1.34	1.79	1.78	1.96
Lys	0.05	0.05	0.07	1.35	0.07
Met	0.03	0.03	0.04	0.46	0.02
Phe	0.84	0.84	1.13	1.20	1.34
Thr	0.69	0.69	1.55	1.01	1.11
Val	0.72	0.72	0.96	0.02	0.02
Ala	1.30	1.30	1.58	1.72	1.77
Asp	0.23	0.23	4.37	1.41	2.83
Cys	0.35	0.35	0.47	0.13	1.87
Glu	3.42	3.42	1.06	2.42	4.80
Gly	1.31	1.31	1.06	0.14	0.03
Pro	1.00	1.00	1.06	1.26	1.38
Ser	1.07	1.07	1.06	0.57	0.29
Tyr	1.59	1.59	1.06	0.06	0.03
Carbohydrate, %					
Starch	42.9	40.6	23.3	26.7	33.9
Ethanol Soluble carbohydrates	6.76	10.28	7.29	7.20	5.87
Neutral detergent fiber	1.41	4.49	24.4	29.8	14.2
Acid detergent fiber	3.93	6.17	19.9	18.8	7.22
Minerals, %					
Ash	8.33	11.5	10.9	10.9	8.10
Calcium	1.26	1.12	0.94	0.96	1.43
Phosphorus	0.51	0.51	0.43	0.44	0.53
Sodium	0.36	0.33	0.26	0.32	0.43
Potassium	0.31	0.36	0.30	0.34	0.43
Magnesium	0.08	0.04	0.08	0.07	0.04

Table 4. Apparent retention of components and apparent metabolizable energy in fruit pomaces fed to broiler chickens without or with multi-enzymes supplement

	Apparent retention of components in pomace diets, %					Pomace AME, Kcal kg DM ⁻¹
	Dry matter	Nitrogen	Crude fat	NDF	Gross energy	
<i>Pomace effects</i>						
Apple	71.6 ^a	34.4 ^c	45.9	-173 ^b	76.1 ^a	3,350 ^a
Blueberry	64.2 ^b	54.8 ^a	58.2	25.1 ^a	66.1 ^c	2,613 ^c
Cranberry	64.8 ^b	57.1 ^a	72.7	29.6 ^a	64.6 ^c	2,394 ^{cd}
Grape	67.3 ^b	45.6 ^b	61.9	17.9 ^a	70.4 ^b	3,008 ^{ba}
SEM	1.45	2.58	7.73	13.02	1.31	202.38
<i>ENZ effects</i>						
-	67.5	52.1 ^a	54.7	-20	68.3	2,677
+	66.5	43.9 ^b	64.7	-30.6	70.3	3,006
SEM	1.02	1.82	5.46	9.19	0.92	143.1
<i>Interactions</i>						
-ENZ						
Apple	72.9	27.9	37.2	-139.0 ^c	75.8	3,308
Blueberry	63.9	55.3	51	20.2 ^a	64.6	2,346
Cranberry	64.1	55.4	66.9	37.0 ^a	63.9	2,277
Grape	65	37.1	63.5	1.5 ^a	68.9	2,776
+ENZ						
Apple	70.3	41.0	54.7	-208.8 ^b	76.4	3,392
Blueberry	64.7	54.5	65.5	30.1 ^a	67.8	2,880
Cranberry	65.4	58.9	78.5	22.2 ^a	65.2	2,512
Grape	69.7	53.9	60.2	34.2 ^a	71.9	3,240
SEM	2.04	3.65	10.93	18.38	1.86	286.21
<i>P-Value</i>						
Pomace	0.002	<0.001	0.119	<0.001	<0.001	0.008
ENZ	0.479	0.002	0.198	0.422	0.129	0.109
ENZ x Pomace	0.369	0.068	0.784	0.045	0.878	0.852

¹The ENZ preparation was added at 500g/ton and contained galactanase, protease, β -mannanase, β -glucanase, xylanase, α -amylase and cellulase activities at 50, 200, 400, 600, 7,000, 2,500, 2,800 U/g of product, respectively (Superzyme EO®, Canadian Bio-systems Inc., Calgary, AB Canada).

^{abc}Within a factor of analyses means with different letter superscript differ, $P < 0.05$.

Table 5. Plasma metabolites in 21-day old broiler chickens fed diets with fruit pomaces without or with fiber degrading enzymes.

Item	Total Protein, g/L	Globulin, g/L	Albumin to Globulin ratio	Alkaline phosphatase, U/L	Bile acid, $\mu\text{mol/L}$	Cholesterol, $\mu\text{mol/L}$	CO ₂ , $\mu\text{mol/L}$	Cl, mmol/L	Ca, mmol/L	P, mmol/L	Na, mmol/L	K, mmol/L
<i>Pomace effects</i>												
Apple	23	13.1	0.77	10,676	23.4 ^a	4.1 ^a	23.2 ^a	115.2	2.5 ^a	2.1	151.3	6.3 ^{ba}
Blueberry	22.4	12.9	0.76	12,794	11.1 ^b	2.8 ^b	20.3 ^b	116.3	2.2 ^c	2.1	148.6	6.1 ^{bc}
Cranberry	22.4	13.1	0.72	12,129	15.6 ^b	3.1 ^b	18.2 ^c	117.1	2.3 ^{bc}	2	147.8	5.8 ^c
Grape	22.7	12.9	0.77	14,578	12.6 ^b	2.9 ^b	21.2 ^b	117.2	2.4 ^{ba}	2	150.9	6.6 ^a
SEM	0.5	0.41	0.04	1,840	1.98	0.14	0.57	0.66	0.05	0.06	0.63	0.13
<i>ENZ effects</i>												
-	23.1	13.5 ^a	0.72	11,175	14.9	3.2	20.5	117.3 ^a	2.4	2	150.2	6.1
+	22.2	12.5 ^b	0.78	13,914	16.5	3.3	20.9	115.6 ^b	2.4	2.1	149.2	6.3
SEM	0.35	0.29	0.03	1,301	1.4	0.1	0.4	0.46	0.04	0.04	0.45	0.09
<i>Interactions</i>												
-ENZ												
Apple	23.8	13.5	0.76	13,968 ^{bac}	21.9	3.98 ^a	23	115.6	2.51	2.0 ^{ab}	150.7 ^{abc}	6.2
Blueberry	22.5	13.6	0.67	10,349 ^{bc}	9.87	2.75 ^b	20.5	116.5	2.25	2.2 ^a	149.2 ^{abc}	6.2
Cranberry	22.8	13.5	0.69	9,382 ^{bc}	15.6	3.23 ^b	18.3	118	2.37	2.0 ^{ab}	148.5 ^{abc}	5.7
Grape	23.4	13.4	0.76	11,000 ^{bac}	12.1	2.86 ^b	20.1	119.2	2.27	1.8 ^b	152.1 ^a	6.4
+ENZ												
Apple	22.3	12.6	0.77	7,384 ^c	25	4.26 ^a	23.3	114.7	2.5	2.2 ^a	151.8 ^{ab}	6.4
Blueberry	22.3	12.1	0.83	15,239 ^{ba}	12.4	2.84 ^b	20	116.1	2.2	1.9 ^{ab}	148.0 ^{bc}	5.9
Cranberry	22.1	12.8	0.74	14,876 ^{ba}	15.5	2.92 ^b	18	116.2	2.3	1.9 ^{ab}	147.2 ^c	6.0
Grape	22	12.4	0.77	18,156 ^a	13	3.07 ^b	22.2	115.1	2.5	2.1 ^{ab}	149.7 ^{abc}	6.7
SEM	0.71	0.57	0.01	2,601	2.81	0.2	0.8	0.93	0.08	0.08	0.9	0.18
P-value												
Pomace	0.800	0.950	0.750	0.510	<0.01	<0.01	<0.01	0.121	0.002	0.105	0.105	<0.01
ENZ	0.060	0.070	0.080	0.140	0.425	0.632	0.475	0.009	0.717	0.362	0.362	0.294

ENZ*Pomace	0.78	0.92	0.35	0.04	0.935	0.459	0.339	0.199	0.292	0.008	0.008	0.247
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¹The ENZ was added at 500g/ton and preparation contained galactanase, protease, β -mannanase, β -glucanase, xylanase, α -amylase and cellulase activities at 50, 200, 400, 600, 7,000, 2,500, 2,800 U/g of product, respectively (Superzyme EO®, Canadian Bio-systems Inc., Calgary, AB Canada).

^{abc}Within a factor of analyses means with different letter superscript differ, P<0.05.

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