Antioxidant Effect of Apple Pomace Diet in Intestinal Lymph Nodes of Weaned Piglets Challenged with *Escherichia coli* Endotoxin

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Abstract
The valorization of agro-industrial by-products represents an important strategy for promoting circular economy and sustainable agri-food systems. Apple pomace, a major by-product of the fruit processing industry, is a rich source of dietary fiber and bioactive compounds, particularly polyphenols, with potential applications in animal nutrition. This study aimed to evaluate the effects of dietary apple pomace inclusion on oxidative stress in weaned piglets challenged with bacterial lipopolysaccharides (LPS). A total of 26 weaned piglets were assigned to four experimental groups: control, LPS-challenged, apple pomace and LPS + apple pomace. After 21 days, oxidative stress markers were assessed in intestinal lymph nodes, including antioxidant enzyme activities (catalase, glutathione peroxidase, superoxide dismutase), total antioxidant capacity (TAC) and biomarkers of lipid, protein, and DNA oxidation. LPS challenge significantly impaired antioxidant defense by decreasing enzyme activities and TAC, while increasing oxidative damage to lipids, proteins, and DNA. Dietary inclusion of apple pomace improved antioxidant enzyme activities and partially restored TAC. Moreover, it significantly reduced LPS-induced oxidative damage, as evidenced by decreased levels of protein carbonyls, TBARS, and 8-oxo-2′-deoxyguanosine. In conclusion, apple pomace inclusion in the diet mitigates oxidative stress and enhances antioxidant status in weaned piglets under inflammatory challenge. These findings support the potential use of apple pomace as a functional feed ingredient in animal nutrition.
Keywords: apple, piglets, pomace, waste

1. Introduction

The valorization of agro-industrial residues it's related to the global trend for a circular economy and a sustainable agri-food systems [1]. Large amounts of waste and by-products are generated as a result of the agriculture production and food processing [2]. When not properly managed, the agro-industrial waste can contribute to environmental pollution and economic inefficiencies [3]. However, the agro-industrial waste can be seen as sources of bioactive compounds that can be used for a wide range of applications, including animal nutrition, food production, pharmaceuticals, bioenergy, and biodegradable materials [4].
Apple pomace is a by-product generated from fruit juice industry produced in large quantities in countries with strong fruit-processing sectors, as for example Romania [5]. Apple pomace is the by-product from juice industry consisting mainly of peels, pulp, seeds [6]. Despite considering the apple pomace as a low-value residue, it is in fact a complex matrix rich in dietary fiber, in particular pectin, cellulose, and hemicellulose, as well as polyphenols, sugars, vitamins and

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minerals [7]. These components make apple pomace a product with nutritional and functional potential.

Due to its valuable composition, the apple pomace was used in bakery and dairy production, biotehnology or generation of renewable energy [8].

Animal nutrition is a promising field for the use of apple pomace, as due to its content in bioactive compounds and fibers, it can be included in the diets of ruminants, pigs, and poultry. Literature data have shown that incorporation of apple pomace into the diet of farm animals improve gut health and can influence digestive processes [9]. Also, the use of apple pomace can lower the feeding costs and can reduce the environmental footprint associated with fruit processing [10]. Several studies have shown that apple pomace has a significant potential to enhance immune response, antioxidant capacity and gut health [11]. The present paper investigates the capacity of a diet containing apple pomace to lower the oxidative stress induced by the exposure to bacteria of weaned piglets.

2. Materials and methods

Experimental design. The feding trial was carried out in the National Research and Development for Biology and Animal Nutrition IBNA Balotești experimental farm on 26 weaned TOPIG hybrid piglets (6-7 piglets/lot) with an initial average weight of 7.26± 0.42 kg, for 21 days. After weaning, the piglets were treated with colistin (3 days) to prevent weaning stress. Experimental activation with bacterial lipopolysaccharides from E. coli (LPS) was done 24 hours before slaughter. The animals were divided into four batches and received the following treatments: Control group (6 piglets/lot fed with complete control feed based on corn and soybean meal); LPS group (6 piglets/lot stimulated with E.coli-LPS and fed with complete control feed); Apple pomace group (6 piglets/lot fed with a diet with 5% apple pomace) LPS + Apple pomace group (6 piglets/lot stimulated with E.coli-LPS and fed a diet with 5% apple pomace. Diets were formulated to fullfil the nutritional requirements for swine (NRC, 2012). Piglets were euthanized in accordance with ethical recommendations (European Regulation and Ethics Committee of the National Institute for Research and Development for Nutrition and Animal Biology, Balotești, Romania). After 21 days the animals were euthanized and intestinal lymph nodes were collected and stored at -80ºC until further analyses.

Effect on the activity of antioxidant enzymes. The activities of antioxidant enzymes (catalase, glutathione peroxidase and superoxide dismutase – CAT, GPx and SOD) were analyzed in intestinal lymph nodes tissue lysates using specific kits (Cayman Chemical Company - Ann Arbor, MI, USA) as already described [12]. The results represent the mean of three independent experiments.

Effect on the lipid peroxidation. Lipid oxidation was assessed in intestinal lymph nodes tissue lysates. TBARS (thiobarbituric acid reactive substances) were measured according to the protocol already published by [12]. Results were expressed as nmol malondialdehyde/mL and represent the mean of three independent experiments.

Effect on the protein oxidation. Protein oxidation was assessed in intestinal lymph nodes tissue lysates and the product of reaction between protein carbonyls and 2,4-dinitrophenyl hydrazine was measured spectrophotometrically according to the protocol already described [12]. Results are expressed in nmol/mg of carbonyl content and represent the mean of three independent experiments.

Effect on the DNA oxidation. DNA oxidation was measured in intestinal lymph nodes tissue lysates by the immune-enzymatic method using DNA Oxidative Damage ELISA Kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to the protocol already described [12]. Results are expressed in pg/ml of 8-hydroxy-2'-deoxyguanosine and represent the mean of three independent experiments.

Statistical analysis. Results data are expressed as means ± standard error of the mean (SEM). Differences among groups were tested using one way ANOVA analysis of the GraphPad Prism 9

software followed by Fisher PSLD test. Statistical significance between the experimental groups were considered at a p-value <0.05.

3. Results and discussion

Apple, as well as apple by-products resulting from processing in the agro-industry are rich in natural antioxidants that can delay the oxidation reaction by interrupting the propagation of free radicals or by inhibiting their formation [13]. In the present paper we have investigated the capacity of a diet containing apple pomace to reduce the oxidative stress in intestinal lymph nodes of weaned piglets challenged with LPS bacterial endotoxin.

The activities of antioxidant enzymes catalase, glutathion peroxidase and superoxid dismutase in intestinal lymph nodes of weaned piglets fed with different experimental diets are presented in Figure1.

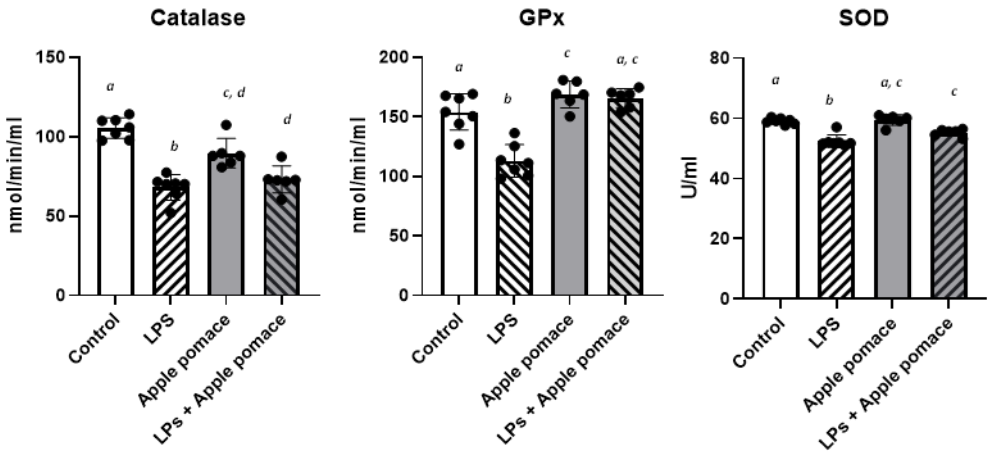

Figure 1. Effect of apple pomace diet on antioxidant enzimes activity

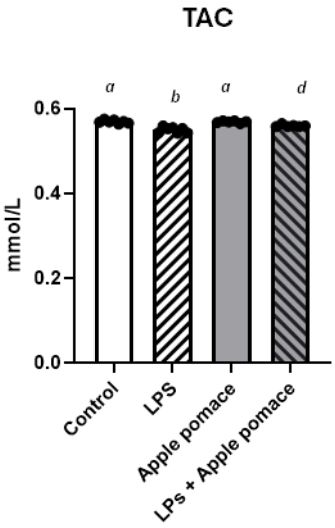

Figure 2. Effect of apple pomace diet on total antioxidant capacity

The activities of antioxidant enzymes were significantly reduced in the LPS group (catalase by 31.9%, GPX by 10.1%, SOD by 11.19%) compared with the control group, confirming that LPS challenge induces oxidative stress and disrupts enzymatic antioxidant defense mechanisms. Previous studies have shown that LPS stimulates production of reactive oxygen species (ROS), impairing thus the endogenous antioxidant systems [14]. Apple pomace diet partially alleviated this effect, as the LPS + apple pomace group showed significantly higher antioxidant enzyme activity - an increase of 46.5% for GPx, 7.3% for CAT and 5.34% for SOD – as compared with LPS group.

The effects of dietary apple pomace supplementation on oxidative stress biomarkers in the intestinal lymph nodes of weaned piglets are presented in Figure 3.

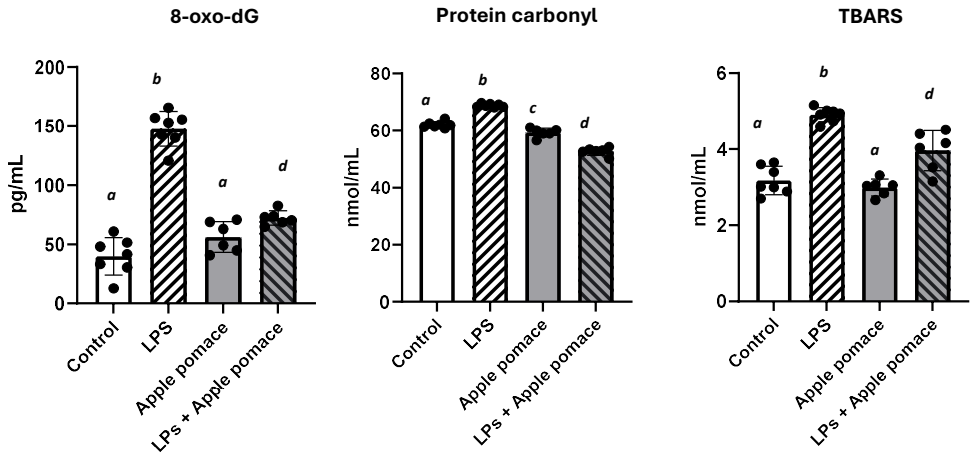

Figure 3. Effect of apple pomace diet on the oxidation of DNA, proteins and lipids.

Total antioxidant capacity (TAC) affected by the experimental treatments was shown in figure 2. LPS induced a significant decrease (3.4%) of TAC in LPS group as compared with the control group, indicating that LPS administration induced systemic oxidative stress. Literature data have shown that LPS stimulates the overproduction of ROS and to outclass the endogenous antioxidant defense system [15]. By contrast, the LPS + apple pomace group showed significantly higher TAC values in intestinal lymph nodes than the LPS group (1.77% increase, $p<0.05$), indicating that apple pomace supplementation partially counteracted the oxidative stress induced by LPS challenge. Similar antioxidant effects of polyphenols from fruits have been reported in animal nutrition studies and can be related to their ability to scavenge free radicals and to stimulate antioxidant enzyme activity [16].

Significant differences among experimental groups were observed for 8-oxo-2′-deoxyguanosine (8-oxo-dG), protein carbonyl, and TBARS concentrations ($p < 0.05$), indicating that both LPS challenge and dietary treatment influenced oxidative damage of DNA, proteins and lipids.

Exposure to LPS significantly increased the concentration of 8-oxo-dG, a marker of oxidative DNA damage, as compared with the control group (3.7 times increase; $p<0.05$), confirming that LPS administration induced oxidative stress and promoted DNA oxidation in the intestinal lymph nodes of weaned piglets. Administration of apple pomace alone did not induce DNA oxidative damage animals as showed by the 8-oxo-dG levels similar to those observed in the control group. By contrast, significantly lower 8-oxo-dG concentrations was observed in LPS + apple pomace group as compared with LPS group (48% decrease; $p < 0.05$), indicating that apple pomace supplementation partially alleviated LPS-induced DNA oxidation. These findings are consistent with literature data showing that dietary polyphenols protect the body against the effects of reactive oxygen species on DNA integrity, when present at low concentrations [17].

Protein oxidation involves the initial modification of amino acid side chain groups and subsequent conversion to reactive derivatives as carbonyls (aldehydes and ketone) as a hallmark of protein oxidation [18]. LPS exposure significantly increase protein carbonyl content in comparison with the control group (10.8% increase, $p < 0.05$), indicating an increase of protein oxidation in lymph nodes tissue under LPS challenge. By contrast, the apple pomace group has a significantly lower protein carbonyl concentrations (59.3nmol/mL) than both the control (62 nmol/mL) and LPS (68.7 nmol/mL) groups, suggesting that dietary apple pomace may enhance antioxidant protection and reduce proteins oxidation. Furthermore, piglets receiving LPS + apple pomace diet exhibited lower protein carbonyl levels than the LPS group (52.7nmol/mL vs 68.7 nmol/mL, $p < 0.05$), demonstrating that apple pomace supplementation can mitigate protein oxidative damage induced by LPS challenge. Other literature data have shown that

broilers fed selenium yeast and polyphenols from algae showed significant inhibition of protein carbonyl formation in the muscle tissues [19].

The concentration of TBARS, representing an indicator of lipid peroxidation, was also significantly affected by the treatments. Lymph nodes tissues from the LPS group of piglets showed significantly higher TBARS levels (54% increase; $p < 0.05$) compared with the control group. In apple pomace group, apple pomace did not promote lipid oxidative damage as TBARS concentrations were similar to those observed in the control group. Apple pomace succed to partially prevent lipid peroxidation to TBARS values (3.96nmoli/mL) between that of LPS group and those of the control and apple pomace groups (4.9nmoli/mL vs 3.17nmoli/mL and 2.99 nmoli/mL, respectively; $p < 0.05$), suggesting that while apple pomace supplementation did not completely prevent lipid peroxidation induced by LPS, it significantly reduced oxidative damage of membrane lipids.

Our results showed that LPS induce an increase of oxidative damage and a decrease of the antioxidant capacity in intestinal lymph nodes of weaned piglets. On the other side apple pomace due to the high content in polyphenols inprove antioxidant status and attenuate the oxidative damage induced by LPS. In conclusion , these results support the potential use of apple pomace as a functional feed ingredient capable of improving antioxidant status in animals exposed to inflammatory or oxidative challenges.

4. Conclusions

Our results showed that LPS induce an increase of oxidative damage and a decrease of the antioxidant capacity in intestinal lymph nodes of weaned piglets. On the other side apple pomace due to the high content in polyphenols improve antioxidant status and attenuate the oxidative damage induced by LPS. In conclusion, these results support the potential use of apple pomace as a functional feed ingredient capable of improving antioxidant status in animals exposed to inflammatory or oxidative challenges.

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