EFFECT OF APPLE POMACE DIET ON BIOCHEMICAL AND ANTIOXIDANT PARAMETERS IN PLASMA OF LPS CHALLENGED WEANED PIGLETS

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Abstract

The valorization of agro-industrial by-products is increasingly recognized as a key strategy for advancing the circular economy and fostering more sustainable agri-food systems. Apple pomace, one of the main by-products generated by the fruit processing industry, contains substantial amounts of dietary fiber and bioactive compounds, particularly polyphenols, making it a promising ingredient for use in animal nutrition. This study aimed to evaluate the effects of dietary apple pomace inclusion on biochemical and antioxidant parameters in plasma of LPS (bacterial lipopolysaccharides) challenged weaned piglets. 26 weaned piglets (6-7 piglets/group) were assigned to four experimental groups: control, LPS-challenged, 5% apple pomace and LPS + 5% apple pomace. After 21 days, markers of the metabolism of proteins (total protein, albumin, bilirubin, creatinine, urea), lipids (cholesterol, triglycerides), sugars (glucose), mineral profile (phosphorus, calcium, magnesium, iron), liver enzymes (ALAT, ASAT, Alkaline Phosphatase and GammaGT), antioxidant enzyme activities (catalase- CAT, glutathione peroxidase- GPx, superoxide dismutase-SOD), total antioxidant capacity (TAC), and biomarkers of lipid oxidation (TBARS) were assessed in plasma of the animals. LPS challenge significantly increased cholesterol, triglycerides, glucose, albumin, bilirubin and creatinine in plasma of LPS challenged piglets group. The activities of hepatic enzymes ALAT, ASAT, Alkaline Phosphatase and GammaGT were increase by LPS challenge, as well as the lipid oxidation (TBARS), while antioxidant enzymes activity (catalase and superoxide dismutase) activities were significantly decreased. Administration of apple pomace in the diet has improved some plasma biochemical parameters as cholesterol, glucose, triglycerides, bilirubin, lipid oxidation, as well as the activities of hepatic enzymes ALAT and gamma GT, and the activities of antioxidant enzymes CAT and SOD. In conclusion, a diet containing 5% apple pomace can improve biochemical parameters and oxidative stress in weaned piglets exposed to bacterial lipopolysaccharide LPS. These findings highlight the potential of apple pomace as a functional feed ingredient with promising applications in animal nutrition.

Key words: *weaned piglets, oxidative stress, apple pomace, biochemical markers*

INTRODUCTION

The valorization of agro-industrial residues has become an important strategy for promoting circular bioeconomy principles and improving the sustainability of agri-food systems. Agricultural production and food-processing activities generate substantial quantities of by-products that, when inadequately managed, may contribute to environmental pollution and the inefficient use of valuable resources. Nevertheless, these materials can be recovered and converted into valuable resources for animal feeding, food production, and biotechnological

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applications, supporting more sustainable production systems [1].

Apple pomace is one of the main by-products generated by the fruit juice processing industry and represents a promising resource for sustainable valorization. It consists primarily of residual apple pulp, peel, and seeds and contains substantial amounts of dietary fibre, including pectin, cellulose, and hemicellulose, as well as polyphenols, sugars, vitamins, and minerals. Its complex nutritional and phytochemical composition has attracted growing scientific interest, highlighting its potential as a functional ingredient in food and feed applications [2, 3]. The recovery and utilization of these constituents may contribute to reducing food-processing waste while increasing the value obtained from agricultural raw materials.

In addition to its applications in food processing and biotechnology, apple pomace has considerable potential in animal nutrition. Its dietary fibre and polyphenolic compounds may influence digestive processes, antioxidant status, and other physiological functions, although the magnitude of these effects depends on the animal species, inclusion level, processing method, and composition of the basal diet. Recent reviews have highlighted the potential of fruit-processing by-products to improve the nutritional value of animal diets, reduce reliance on conventional feed ingredients, and support more sustainable livestock production [3,4]. Experimental evidence in dairy cattle has also demonstrated that dried apple pomace can modify antioxidant-related parameters in ruminal fluid, although the observed responses were not uniformly beneficial across all measured markers [5].

The potential of apple pomace to modulate oxidative balance provides a rationale for investigating its use in the diets of weaned piglets, a production stage characterized by physiological and environmental challenges that may compromise intestinal function and antioxidant defence. However, evidence regarding its protective effects against bacteria-induced oxidative stress in weaned pigs remains limited. Therefore, the present study aimed to investigate whether dietary supplementation with apple pomace could attenuate oxidative stress associated with bacterial exposure in weaned piglets, contributing to the assessment of its potential as a functional feed ingredient and supporting the sustainable valorization of fruit-processing by-products.

MATERIAL AND METHOD

Experimental design. The feeding trial was conducted at the experimental farm of the National Research and Development Institute for Animal Biology and Nutrition (IBNA), Balotești, Romania, using 26 weaned TOPIG hybrid piglets (6-7 animals/group) with an initial average body weight of 7.26 ± 0.42 kg. The experimental period lasted 21 days. Following weaning, the piglets received colistin treatment for three days as part of the post-weaning management protocol. To induce an inflammatory challenge, the animals were exposed to *Escherichia coli* lipopolysaccharide (E. coli LPS) 24 hours before slaughter. The piglets were assigned to four experimental groups according to dietary treatment and LPS exposure: (1) NC-Control, the control group, receiving a standard complete diet based on corn and soybean meal; (2) NC-E1, the LPS-challenged group, receiving the standard control diet; (3) NC-E2, the apple pomace group, receiving a complete diet supplemented with 5% apple pomace; and (4) NC-E3, the apple pomace + LPS group, receiving a diet containing 5% apple pomace and subjected to the LPS challenge. At the end of the 21-day experimental period, the piglets were euthanized in accordance with applicable animal welfare regulations and the ethical requirements

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approved by the relevant institutional ethics committee at IBNA, Balotești, Romania. Blood samples were collected from jugular vein in Li-heparin tubes in order to obtain plasm after blood centrifugation. Samples were stored at -80 °C until further biochemical analyses.

Assessment of plasma parameters. The effect of feed containing apple pomace on the general health status - including blood glucose, lipid profile (total cholesterol and triglycerides), protein profile (total protein, albumin, bilirubin, creatinine, urea), hepatic markers (aspartate aminotransferase (AST) alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), and electrolytes in blood plasma collected at the end of the experiment - was evaluated using a PENTRA 400 biochemistry analyzer. A complete blood count (hemogram) was determined using an MS 4S Veterinary hematology analyzer.

Assessment of antioxidant enzyme activity. The activities of the antioxidant enzymes catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were determined in plasma samples using specific assay kits from Cayman Chemical Company (Ann Arbor, MI, USA), following the procedure previously described by Marin et al. [14] The results are presented as the mean of three independent experiments.

Assessment of lipid peroxidation. Lipid peroxidation was evaluated in plasma samples by measuring thiobarbituric acid-reactive substances (TBARS), following the protocol described by Marin et al. [14. The results were expressed as nanomoles of malondialdehyde per millilitre (nmol MDA/mL) and represent the mean of three independent experiments.

Measurement of Plasma Total Antioxidant Capacity. Plasma total antioxidant capacity (TAC) was determined using a QuantiChrom Total Antioxidant Capacity Assay Kit (BioAssay Systems, Hayward, CA, USA), according to the manufacturer's instructions. Briefly, 20 µL of undiluted plasma or Trolox standard solution was added to a 96-well microplate, followed by 100 µL of working reagent. The reaction mixture was mixed by gently tapping the plate and incubated at room temperature for 10 min. Absorbance was then measured at 570 nm using a Tecan Infinite M200 microplate reader (Tecan Group Ltd.). Plasma TAC values were calculated based on the Trolox standard curve and expressed as µM Trolox equivalents.

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.

RESULTS

Effect of the diets on plasma lipid profile and glycemia. Plasma cholesterol, triglyceride and glucose concentrations were significantly affected by the experimental treatments (Figure 1).

Compared with the control group, LPS administration resulted in significantly increase of the concentration of cholesterol (37.9%), glucose (28.7%), tryglicerides (60.7%). Dietary inclusion of apple pomace, in the absence of LPS stimulation, maintained cholesterol, triglyceride and glucose concentrations at levels comparable to those of the control group. In the LPS+Apple pomace group, all three parameters remained significantly lower than in LPS group with decrease of 13.15% for glucose), 13.2% (for cholesterol) and 22.19% (for tryglicerides), but with values higher than in control group.

Effect of the diets on plasma proteins and indicators of hepatic and renal

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function. Total protein, albumin, bilirubin, creatinine and urea were assessed to characterize the systemic biochemical response to the experimental treatments and the results are presented in the figure 2.

LPS exposure induced a significant increase of albumin (by 1.52 times), bilirubin (by 1.33 times), creatinine (by 1.14 times), while reducing the total protein concentration by 1.10 times as compared with the control. Urea concentrations in plasma were not affected by the treatements. While apple pomace doesn't affect the concentration of plasma proteins and the indicators of hepatic and renal function, in the presence of LPS stimulation, apple pomace diet can significantly alleviate the concentrations of bilirubin and creatinine, but have no effect on albumin or contrary a sinergic effect with LPS on plasma total protein concentration.

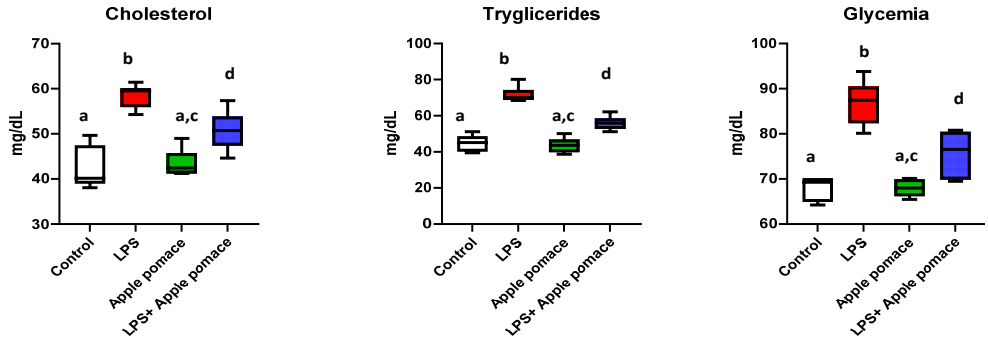


Fig. 1. Effect of the diets on plasma lipid profile and glycemia

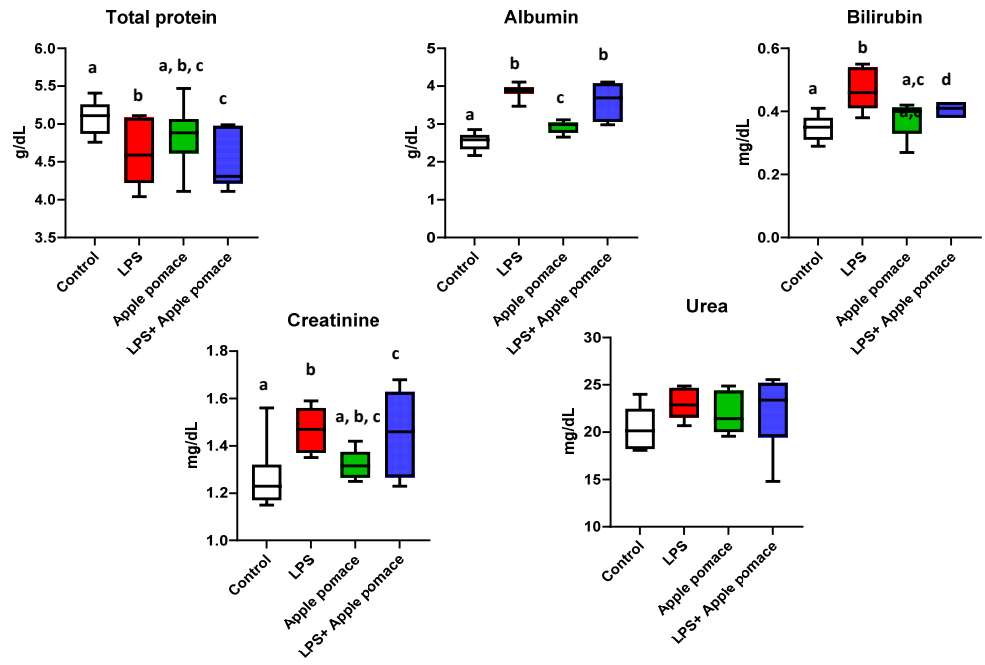


Fig. 2. Effect of the diets on plasma proteins and indicators of hepatic and renal function

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Effect of the diets on plasma mineral concentrations. Plasma phosphorus and calcium concentrations did not differ significantly among the experimental groups (Figure 3), indicating that neither LPS stimulation nor dietary apple pomace produced detectable changes in the circulating concentrations of these minerals during the experimental period.

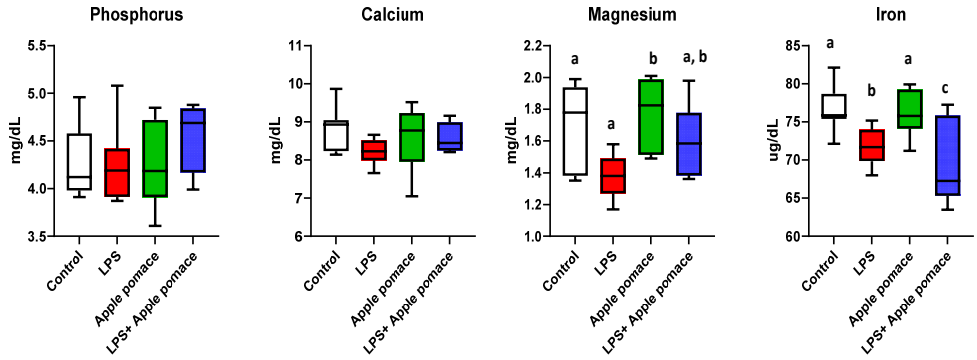


Fig. 3. Effect of the diets on plasma mineral concentrations

Iron concentrations were significantly lower in the LPS group (P<0.05) than in the control group. The Apple pomace group showed plasma iron concentrations statistically comparable to those of the control group, whereas the LPS+Apple pomace group exhibited the lowest distribution and differed significantly from all other groups (68.49mg/dL vs 76.91 mg/dL in Control group and 71.49 mg/dL in LPS group).

Effect of the diets on plasma enzyme activities associated with hepatic function. The activities of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) are presented in Fig. 4. LPS administration significantly increased hepatic enzymes activities compared with the control group: 56% increase for ALAT, 67.88% for ASAT, 65% for ALP and 60.7% for GGT. The Apple pomace group showed an activity comparable to that of the control group, whereas the LPS+Apple pomace group displayed an intermediate value that was statistically significant different from LPS group for ALAT and GGT.

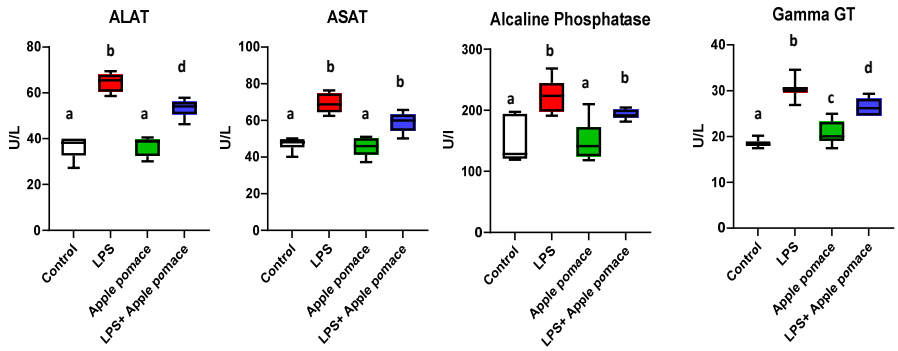


Fig. 4. Effect of the diets on plasma enzyme activities associated with hepatic function

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Effect of the diets on plasma antioxidant enzyme activities. The activities of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) were evaluated in order to characterize the antioxidant response of the piglets (Fig. 5).

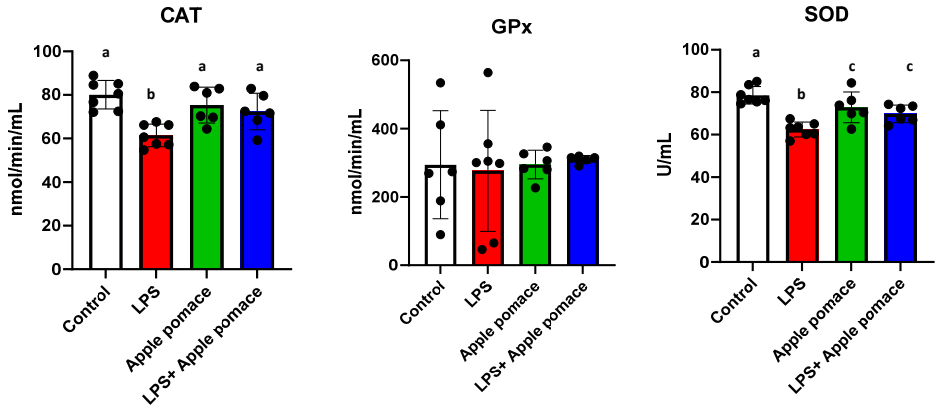


Fig. 5. Effect of the diets on on plasma antioxidant enzyme activities

CAT and SOD activities were significantly lower in the LPS group (61.44 nmol/min/mL and 62.44 U/mL) than in the control group (80.07 nmol/min/m L and 78.58 U/mL, respectively). In contrast, CAT and SOD activities in both the Apple pomace and LPS+Apple pomace groups was statistically comparable to that in the control group. There were no significant differences in GPx activities, regardless of the treatment group.

Effect of the diets on total antioxidant capacity and lipid peroxidation. Plasma total antioxidant capacity (TAC) and thiobarbituric acid-reactive substances (TBARS) were measured to provide complementary information on antioxidant status and oxidative damage (Figure 6). TBARS had a significantly higher concentration in LPS group than in control, while TAC was not significantly different between the two groups. The Apple pomace group displayed values statistically comparable to the control. Interestingly, TBARS in the LPS+Apple pomace group has a value intermediate between control and LPS groups.

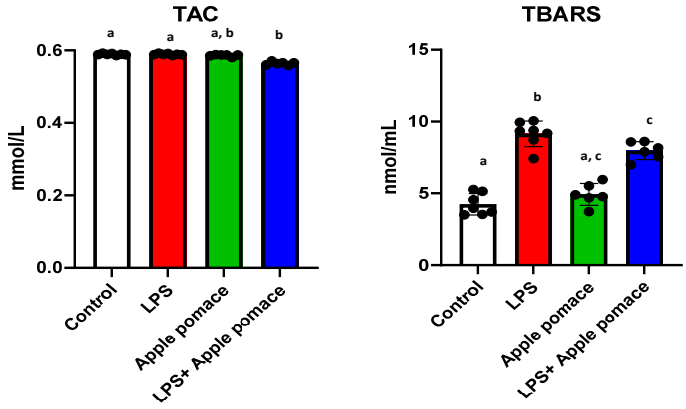


Fig. 6. Effect of the diets on total antioxidant capacity and lipid peroxidation

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DISCUSSION

The weaning crisis in piglets is a critical physiological and immunological challenge caused by the abrupt shift from liquid milk to solid feed, environmental changes, and social stress [7]. This transition triggers severe intestinal inflammation and oxidative stress [8]. Agro-industrial and food waste processing by-products are highly effective alternatives for managing the post-weaning crisis in piglets. Fruit pomaces are valuable sources of bioactive compounds, including dietary fiber, vitamins, and polyphenols, which support their potential application as ingredients in food and animal feed [9, 10]. In piglet nutrition, apple pomace has been investigated as a dietary ingredient to mitigate the adverse effects of weaning, reduce the incidence of diarrhea, and improve growth performance [11, 12]. Similarly, the dietary inclusion of dried apple pomace has been associated with improved feed conversion efficiency and a more favorable fatty acid profile in pork [13]. Dried fruit and vegetable pomaces retain substantial amounts of biologically active compounds, although their nutritional composition may vary depending on several factors. Our previous research demonstrated that apple cultivar significantly influences the concentrations of dietary fiber, total polyphenols, epicatechin, and selected minerals in apple pomace [14]. Moreover, in porcine lymph nodes, apple pomace diet administration significantly reduced LPS-induced oxidative damage, as evidenced by decreased levels of protein carbonyls, TBARS, and 8-oxo-2′-deoxyguanosine [5]. Furthermore, the incorporation of dried fruit pomace into animal diets may enhance the bioflavonoid content of meat, improve growth performance, and alleviate oxidative stress in pigs [15]. These findings highlight the potential of fruit and vegetable pomaces as functional feed ingredients, contributing to the valorization of agro-industrial by-products while supporting animal health and product quality.

The 21-day experiment showed that LPS stimulation was associated with changes in several plasma biochemical indicators in weaned piglets, including cholesterol, triglycerides, glucose, bilirubin, creatinine, iron, hepatic-associated enzyme activities, CAT and SOD activities, and TBARS concentrations. Literatura data shows that the exposure to LPS, triggers a robust systemic inflammatory response in swine that heavily impacts the liver. Thus, LPS exposure causes an increase in liver total cholesterol and LDL-cholesterol concentrations, alongside a marked elevation of hepatic-associated enzyme activities (such as AST, ALT, and ALP) in the serum [16]. These alterations reflect acute liver injury, cellular membrane leakage, and a pronounced disruption of hepatic lipid metabolism.

Dietary inclusion of apple pomace alone generally maintained the lipid profile, glycemia and several enzyme activities at levels comparable to those of the control group. It was also associated with lower TBARS concentrations than those observed in the LPS group, suggesting a potential beneficial effect on the plasma oxidative status. However, the responses in the LPS+Apple pomace group were heterogeneous. Some parameters, particularly CAT and SOD activities, but also hepatic enzymes as ALAT and GGT showed partial recovery relative to LPS alone, while TBARS concentrations were reduced but remained above control values. Other parameters, including ASAT, ALP and TAC, showed patterns indicating that the combined treatment did not consistently restore the control profile.

CONCLUSIONS

In conclusion, dietary apple pomace showed potential to modulate selected plasma biochemical and oxidative stress indicators in weaned piglets subjected to

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LPS stimulation. The effects were parameter-specific, and further evidence is needed to establish the mechanisms involved and the extent to which these changes translate into improved physiological resilience during the post-weaning period.

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