Scientific Papers. Series D. Animal Science. Vol. LXIX, No. 1, 2026
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

DIET WITH APPLE POMACE IS ABLE TO COUNTERACT OXIDATIVE STRESS IN SPLEEN OF PIGLETS EXPERIMENTALLY CONTAMINATED WITH *Escherichia coli* LIPOPOLYSACCHARIDE DURING POST-WEANING PERIOD

Iulian Alexandru GROSU, Ionelia TĂRANU, Gina Cecilia PISTOL, Ana Maria CIUPITU, Daniela MARIN

National Research-Development Institute for Animal Biology and Nutrition (INCDBNA-IBNA), 1 Calea Bucuresti, 077015, Balotesti, Romania

Corresponding author email: grosu.iulian@ibna.ro

Abstract

The spleen plays an essential role in the immune and circulatory system, contributing to the body's defense against infections through the filtering of blood-borne pathogens and antibody production. Oxidative stress, the imbalance between free radicals and antioxidants can affect spleen causing inflammation, cell damage and at the long-term different spleen disorders. In the present study we investigated the effect of a diet including apple pomace to mitigate the oxidative stress and the impairment of antioxidant enzymes (catalase-CAT, superoxide dismutase-SOD and glutationperoxidase-GPx) activity in piglets after weaning experimentally challenged with E. coli lipopolysaccharide (LPS), a well-known inducer of oxidative stress and inflammation. Twenty-four piglets were randomly allocated to four experimental diets: group 1 and 2 received a normal diet for postweaning period while group 3 and 4 received a diet including 5% of apple pomace. In the day 20 with 24h before slaughtering the groups 2 and 4 were challenged with LPS at 80µg/kg b.w. In the day 21 piglets were slaughtered and samples of spleen were collected to determined oxidative and antioxidative stress markers. Results showed that E. coli LPS produced a significant increase of lipid, protein and DNA oxidation and reduce antioxidant enzymes activity. Apple pomace alone did not stimulate splenic antioxidant enzyme activities, but in LPS-challenged piglets, the diet with apple pomace attenuated partially the reduction in SOD, CAT, and GPx activity as well as the increase of oxidative markers, suggesting a protective effect on antioxidant defences under inflammatory oxidative conditions.

Key words*: apple pomace, oxidative stress, piglets, post-weaning.*

INTRODUCTION

The post-weaning period is widely recognized as a critical biological physiological stage in pig production, marked by abrupt separation from the sow, dietary and environmental change, social mixing, and immature digestive and immune function (Taranu et al., 2022). These converging stressors frequently impair feed intake and gut barrier integrity, favour dysbiosis, and increase susceptibility to opportunistic infections, with downstream consequences that include systemic inflammation and oxidative stress (Cui et al., 2024; Dufourny et al., 2021; Yan et al., 2024).

Uncontrolled oxidative stress with an overproduction of free radicals (ROS) that overcomes the body's antioxidant defense system is a key element of post weaning inflammation and must be taking into account when formulating nutritional strategies for this period (Huang et al., 2022; C. Li et al., 2020).

Within this context, the spleen is a relevant target organ. Beyond its classical hematological function (hematopoietic organ), the spleen is a central secondary lymphoid tissue that integrates innate and adaptive immune responses, filters blood-borne antigens, and supports immune cell activation and antibody production (Pistol et al., 2023). In young animals when exposed to inflammatory stimuli, splenic redox homeostasis is especially important because excessive oxidative pressure can compromise immune cell viability and function, promote macromolecular damage, and amplify inflammatory signaling. Key enzymatic defense in this system includes superoxide dismutase, catalase, and glutathione peroxidase,

which act sequentially to detoxify superoxide and hydrogen peroxide and to limit propagation of lipid and protein oxidation (Huang et al., 2023; Y. Li et al., 2020). Measuring these enzymes therefore provides a functional readout of the spleen's capacity to buffer oxidative challenge during the post-weaning transition.

Interest in agroindustry by-products as functional feed ingredients has increased in parallel with circular-economy objectives, and apple pomace is a prime example (Van Zanten et al., 2019; Yafetto et al., 2023). Generated in large amounts by juice and cider processing, apple pomace retains substantial quantities of bioactive compounds, including polyphenols, dietary fibre, and residual micronutrients (Anghel, 2024; Ao et al., 2022; Dufourny et al., 2021; Taranu et al., 2023). These components have been associated with modulation of gut microbiota, reinforcement of intestinal barrier function, and attenuation of inflammatory tone, all of which are highly relevant to post-weaning physiology (Ao et al., 2022; Dufourny et al., 2021; Guo et al., 2022; Taranu et al., 2023). From a redox perspective, apple-derived polyphenols are reported to directly scavenge reactive species and, importantly, to influence cellular antioxidant programs that govern enzymatic defense (Huang et al., 2022). Across animal models and feeding studies, apple pomace or apple polyphenol preparations have been linked to improved antioxidant status, including increases in superoxide dismutase and glutathione peroxidase activity and/or preservation of catalase activity under stress conditions, accompanied by reduced indices of lipid peroxidation (Ao et al., 2022; Chedea et al., 2019; Huang et al., 2023).

Other reports show that pomace and polyphenol-based interventions can normalize antioxidant enzyme activities when they are suppressed by challenge conditions rather than indiscriminately stimulating them in healthy tissue, a pattern consistent with a stress-mitigating, homeostasis-restoring role (Cui et al., 2024; Q. Li et al., 2020; Y. Li et al., 2020). These observations provide a rationale for focusing on superoxide dismutase, catalase, and glutathione peroxidase as sensitive endpoints when evaluating apple pomace in weaned piglets.

Lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria, is a well-established trigger of systemic innate immune activation and oxidative stress through pathways that enhance cytokine production, increase reactive oxygen species generation, and disrupt antioxidant enzyme function (Bonetti et al., 2024; C. Li et al., 2020). The LPS challenge is relevant to the post-weaning stage, when piglets face heightened exposure to enteric pathogens and endotoxin translocation risk due to barrier instability (Yan et al., 2024; Zhang et al., 2021). LPS has been used experimentally to mimic weaning stress and to study different possibilities for countering it. Therefore, evaluating dietary apple pomace against an acute *E. coli*-LPS challenge allows assessment of whether this agri-food by-product can preserve splenic antioxidant defences and limit oxidative damage in a setting that mimics key elements of post-weaning inflammatory pressure.

MATERIALS AND METHODS

Animals and Diets

Twenty-four TOPIGS-40 weaned piglets (21 days of age; initial body weight 7.26 ± 0.42 kg), individually identified by ear tags, were randomly allocated to four experimental groups (n = 6/group) and maintained on their respective dietary treatments for 21 days. The first group (Control group) received a standard complete diet based on corn and soybean meal, according to NRC (2012) for the weaning period. The second group (LPS group) received the same control diet and was challenged intraperitoneally with *E. coli*-LPS at a dose of 80µg/kg body weight, 24h before slaughter (day 20). The third group (apple pomace group-AP) fed a complete diet including 5% apple pomace obtained from a local apple juice producer from Voinești, Dâmbovița County, Romania. The fourth group (apple pomace plus LPS group-AP+LPS) received the same 5% apple pomace diet and was likewise challenged intraperitoneally with LPS (80 µg/kg body weight) 24 h before slaughter.

The proximate composition (dry matter, crude protein, fat, crude fibres and ash were determined with ISO methods (SR EN ISO 5983-2:2009; SR ISO 6492:2001; SR EN ISO

6865:2002: SR EN ISO 2171: 2010), using the semiautomatic Kjeltec Auto 2300 system); minerals were performed by atomic absorption spectrometry after microwave digestion and mineralization with nitric acid (Regulation (EC) no. 152/2009 STAS 9597/16-86STAS 9597/17-86).

Animals were housed at the experimental facility of the National Research-Development Institute for Animal Biology and Nutrition, Balotești, Romania, with two pens assigned to each treatment group (3 piglets/pen).

At the end of the 21-day experimental period, piglets were fasted and euthanized by electrically stunned in compliance and spleen samples were collected on ice and stored at –80°C until analysis. All procedures were conducted with efforts to minimize suffering at slaughter with applicable ethical standards and regulations (EU Council Directive 98/58/EC, Romanian Law 43/2014, and approval of the Ethics Committee of the National Research and Development Institute for Animal Biology and Nutrition, Balotești, Romania).

Determination of polyphenols content of Apple Pomace

Polyphenols from apple pomace were extracted with 80% acetone using a sample-to-solvent ratio of 1:7 (w/v) at 37°C under continuous shaking (Thermo Fisher, Biosan, USA) for 20h. Total polyphenols concentration was then quantified from acetonic extract by using an UV-Vis Spectrophotometer Specord 205 (Analytik Jena, GMbH, Germany) and Folin-Ciocalteu reagent. The absorbance was read at 750nm and the concentration was calculated on a gallic acid curve calibration (r^2 =0.9985) as described by Chedea et al., 2017. The results are expressed as gallic acid equivalent (GAE)/g of apple pomace.

The polyphenolic profile (individual polyphenolic compounds) of the acetone extract was characterized by high-performance liquid chromatography (HPLC) using a Jasco Chromatograph (Jasco Corporation, Tokyo, Japan) equipped with Lichrosorb RP-C18 column (25 x 0.46 cm) and UV/Vis detector, based on retention times and UV detection (270 nm) as described by Vlassa et al., 2022 (Vlassa et al., 2022). Results are expressed as mg gallic acid equivalent per 100 ml extract (mg GAE/ml). All determinations were performed in triplicates.

Determination of total antioxidant capacity

Total antioxidant capacity (TAC) was assessed using an ABTS radical cation-based assay namely 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) as described by Marin et al. 2017 (Marin et al., 2017). Absorbance was recorded at 732 nm immediately after H_2O_2 addition (A0) and again after 3 min of incubation at 37°C (A3) using a Uvikon 923 double-beam spectrophotometer (Kontron Instruments, Zurich, Switzerland). The percentage inhibition of ABTS radical cation formation was converted to Trolox equivalent antioxidant capacity (TEAC) using a Trolox calibration curve (0-2.5 mM). Results were expressed as mM TEAC/g and were used as an index of the sample antioxidant capacity.

Antioxidant Enzyme Activity

The activities of the antioxidant enzymes glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) in spleen tissue were determined using commercial assay kits from Cayman Chemical Company (Ann Arbor, MI, USA), as previously described (Marin et al., 2024). GPx and CAT activities were expressed as nmol/min/mL, while SOD activity was expressed as U/mL.

Assessment of Lipid Peroxidation

Lipid oxidation was assessed in spleen tissue lysates prepared in PBS, as previously described (Marin et al., 2024). Briefly, 200 µL of tissue lysate was mixed with 26 µL of 0.5 N HCl and 260 µL of TBA-TRIS reagent, then incubated at 95°C for 60 min. The reaction was terminated by cooling the samples on ice for 10 min, and absorbance was measured at 532 nm using a Varioskan™ LUX microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Results were expressed as nmol/mL thiobarbituric acid-malondialdehyde (TBARS-MDA).

Assessment of Protein Oxidation

Protein oxidation was evaluated spectrophotometrically (560 nm excitation wavelength and 585-595 nm emission wavelength) by measuring protein carbonyls following derivatization with 2,4-dinitrophenylhydrazine, according to the protocol described in Marin et al., 2024 (Marin et al., 2024). Results were expressed as nmol carbonyls/mg protein.

Assessment of DNA Oxidation

DNA oxidation was determined by an immunoenzymatic assay using a DNA/RNA Oxidative Damage ELISA kit (Cayman Chemical Company, Ann Arbor, MI, USA) as described by Marin et al. (Marin et al., 2017). Absorbance was measured at 420 nm, and results were expressed as pg/mL 8-hydroxy-2′-deoxyguanosine (8-OHdG).

Statistical analysis

For spleen biochemical analyses, the individual piglet was considered the experimental unit. Data are presented as mean ± standard error of the mean (SEM). Statistical differences among experimental groups were evaluated by one-way analysis of variance (ANOVA) for all analysed parameters. When a significant overall treatment effect was detected by ANOVA ($p < 0.05$), post hoc pairwise comparisons between group means were performed using Tukey's multiple comparisons test in GraphPad Prism (GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

Polyphenolic concentration in apple pomace

The result of the Folin Ciocalteu analysis revealed a concentration of 4.80 mg total polyphenols /100 g of apple pomace. Table 1 showed that the pomace is rich in catechin with anti-inflammatory and antioxidant role, ferulic acid, sinapic acid and luteoil known for their calming inflammatory and antioxidant role as well as the neuroprotective action.

Table 1. Polyphenolic composition of the investigated apple pomace

Polyphenolic compounds	Total Polyphenols (mg GAE/100 g)
Galic acid	0.48
Catechin	2.55
Chlorogenic acid	1.14
Vanillic acid	0.68
Caffeic acid	1.35
Syringic acid	0.16
p-Coumaric acid	0.95
Ferulic acid	10.61
Sinapic acid	4.38
Rutin	0.00
Quercetin	0.00
Luteolin	3.13
TOTAL	25.44

No significant differences were recorded between the control and experimental feed in terms of dry matter, crude protein, crude fat, cellulose and ash. But, the dietary inclusion of apple pomace increased by 19.9% the ADF fiber level and minerals (+8.5% Fe, +10.8% Zn and +11.36% Mn), compared to the control diet. Polyphenols such as caffeic acid (15.57 vs 0112 mg % g), coumaric acid (16.88 vs 0.34 mg % g), ferulic acid, sinapic acid, Rutin, Quercetin, Luteolin were also significantly increased in the diet containing apple pomace.

Effects of Dietary Apple Pomace and LPS Challenge on Splenic Antioxidant Enzyme Activity

The activities of glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) were evaluated in spleen tissue from all experimental groups (Figure 1). Overall, *E. coli* LPS challenge was associated with reduced antioxidant enzyme activity, while dietary apple pomace modulated these responses attenuating the reduction under inflammatory conditions.

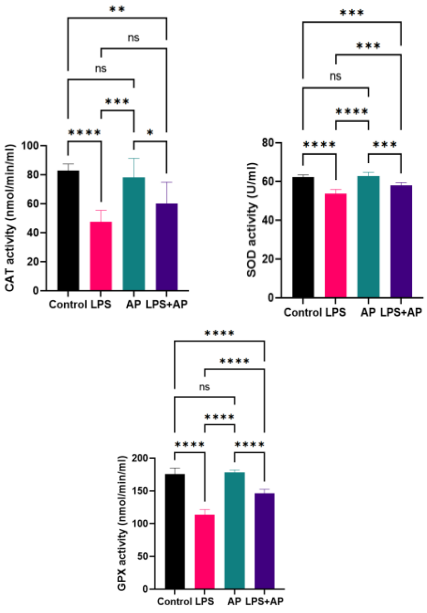

Figure 1. Effects of dietary apple pomace and LPS challenge on splenic antioxidant enzyme activities (CAT, SOD, and GPx) in piglets after weaning. They were fed for 21 days with a Control diet (Control and LPS groups) or AP diet (AP and LPS+AP groups). On day 20, piglets from LPS and LPS+AP groups were challenged with 80 µg LPS/b.w. The results are displayed as mean ± SEM. *=indicates a significant difference ($p < 0.05$); "ns" indicates no significance ($p > 0.05$)

Compared with the Control group, LPS administration significantly reduced CAT activity by 42.69% ($p < 0.0001$). Although, in the AP+LPS group, apple pomace managed to increase CAT activity over that of the group LPS (26.95%), this increase did not reach statistical significance in the Tukey post hoc test ($p = 0.1626$), probably due to the pronounced variability of the values. However, CAT activity in the AP+LPS group remained significantly lower than in the Control group ($p = 0.0047$) and the AP group ($p = 0.0346$). No significant difference was observed between the Control and AP groups ($p = 0.8676$).

A similar overall pattern was observed for SOD activity. Relative to the Control group, LPS significantly decreased SOD activity by 13.88% ($p < 0.0001$). In contrast to CAT, apple pomace inclusion significantly attenuated the LPS-induced decrease in SOD, with SOD activity in the AP+LPS group being 8.25% higher than in the LPS group ($p = 0.0006$). Nevertheless, SOD activity in the AP+LPS group remained significantly lower than in both the Control ($P = 0.0010$) and AP ($p = 0.0005$) groups. SOD activity did not differ between the Control and AP groups ($p = 0.9629$).

GPx activity was also significantly affected by LPS challenge, showing a 35.37% decrease in the LPS group compared with the Control group ($p < 0.0001$). Dietary apple pomace significantly attenuated this reduction, as GPx activity in the AP+LPS group was 28.79% higher than in the LPS group ($p < 0.0001$). However, GPx activity in the AP+LPS group remained significantly lower than in the Control and AP groups (both $p < 0.0001$). No significant difference was observed between the Control and AP groups ($p = 0.9266$).

Taken together, these results indicate that the diet with apple pomace alone did not significantly modify splenic GPx, CAT, or SOD activity under basal conditions, while under LPS-induced oxidative stress it significantly improved SOD and GPx activity, with a non-significant upward trend for CAT.

The post-weaning period is a critical stage in piglets, during which dietary transition, social stress, and environmental changes can increase inflammation and oxidative stress, particularly under the effect of pathogenic endotoxins. Apple pomace is a relevant functional agri-food by-product because it provides polyphenols and fibre with potential redox- and inflammation-modulating effects, while also supporting circular feed-use strategies (Van Zanten et al., 2019). The *E. coli*-LPS challenge used here was deemed appropriate to test whether dietary apple pomace could preserve splenic antioxidant defences under acute inflammatory oxidative stress, as suggested by previous work on apple-derived polyphenols and other polyphenol-rich by-products in piglets (Guo et al., 2022; Huang et al., 2022; Pistol et al., 2023).

In the present study LPS suppressed splenic GPx, CAT, and SOD activities, indicating impaired enzymatic antioxidant defences in the spleen during post-weaning inflammatory stress.

Because these enzymes act sequentially to neutralize reactive oxygen species and limit oxidative damage, their reduction reflects diminished antioxidant buffering capacity. This pattern is consistent with LPS-challenged piglet studies reporting decreased antioxidant enzyme activity together with increased oxidative stress markers (Cui et al., 2024; Huang et al., 2023; Xu et al., 2019). Apple pomace alone did not significantly alter splenic GPx, CAT, or SOD activity under basal conditions, whereas under LPS challenge it improved SOD and GPx activity and showed a non-significant upward trend for CAT. This indicates a stress-responsive protective effect, which is compatible with the concept that polyphenol-rich ingredients preserve enzyme function under oxidative challenge by lowering reactive oxygen species burden and supporting redox-sensitive pathways. The partial recovery observed here (significant for SOD and GPx, weaker for CAT) is suggestive given the tissue- and enzyme-specific nature of antioxidant responses and aligns with reports of organ-specific pomace effects and selective improvements in LPS models depending on tissue and sampling time (Chedea et al., 2019; Huang et al., 2023; C. Li et al., 2020; Xu et al., 2019).

This protective effect is compatible with the polyphenol-rich composition of apple pomace and with evidence that apple polyphenols can enhance antioxidant capacity through direct radical-scavenging and redox-regulated mechanisms; although signalling pathways were not measured here, the preservation of splenic

SOD and GPx activity is consistent with findings reported for apple polyphenols, fermented apple pomace, and related by-product-based antioxidant strategies (Ao et al., 2022; Guo et al., 2022; Huang et al., 2022; Vlassa et al., 2022).

Effects of Dietary Apple Pomace and LPS Challenge on Splenic Lipid Peroxidation (TBARS-MDA)

Splenic lipid peroxidation, assessed by TBARS-MDA, was significantly affected by *E. coli*-LPS challenge and modulated by dietary apple pomace inclusion (Figure 2). Compared with the Control group, LPS administration significantly increased TBARS levels by 66.84% ($p < 0.0001$), indicating enhanced lipid peroxidation under inflammatory oxidative stress conditions. Dietary apple pomace significantly attenuated the LPS-induced increase in TBARS-MDA. In the AP+LPS group, TBARS-MDA levels were 26.44% lower than in the LPS group ($p < 0.0001$), suggesting that apple pomace attenuates partially lipid oxidation damage in the spleen of piglets that received the pomace.

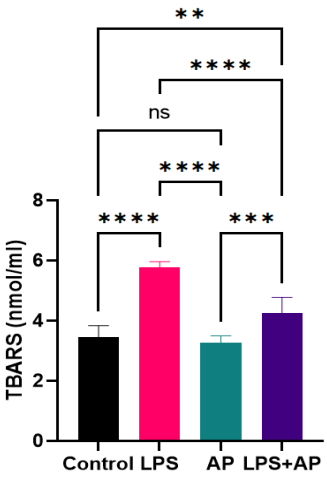

Figure 2. Effects of dietary apple pomace and LPS challenge on splenic TBARS-MDA marker in piglets after weaning. They were fed for 21 days with a Control diet (Control and LPS groups) or AP diet (AP and LPS+AP groups). On day 20, piglets from LPS and LPS+AP groups were challenged with 80µg LPS /b.w. The results are displayed as mean ± SEM. *= indicates a significant difference ($p < 0.05$); "ns" indicates no significance ($p > 0.05$)

However, TBARS values in the AP+LPS group remained significantly higher than those in both the Control group ($p = 0.0028$) and the AP group ($p = 0.0004$).

The diet with apple pomace alone (AP group) did not significantly affect TBARS levels compared with the Control group ($p = 0.7765$). Lipid peroxidation is a key downstream consequence of oxidative stress, as reactive oxygen species attack polyunsaturated fatty acids of cellular membrane and generate secondary products such as TBARS/MDA. The increase in splenic TBARS after LPS challenge indicates measurable lipid oxidative injury beyond enzymatic antioxidant suppression, consistent with reports in weaned piglet challenge models where LPS elevates TBARS/MDA in plasma and/or tissues (He & Wang, 2026; Huang et al., 2023; Q. Li et al., 2020). Apple pomace significantly reduced TBARS in LPS-challenged piglets, but values remained above Control and AP, indicating partial protection. This response is supported by studies showing reduced lipid peroxidation following antioxidant dietary supplementation in LPS-related piglet models, including rice bran oil, benzoic acid plus essential oils, postbiotics plus essential oils, and olive oil cake extract (He & Wang, 2026; Yan et al., 2024; Zhang et al., 2021). Fruits pomace evidence is also relevant, grape pomace reducing lipid peroxidation in piglet organs, (Chedea et al., 2019) and fermented apple pomace improving antioxidant indicators and reduced plasma MDA in weaned pigs (Ao et al., 2022; Vlassa et al., 2022). The reduction in TBARS may reflect direct radical scavenging by apple pomace polyphenols and indirect preservation of antioxidant defenses, limiting propagation of lipid peroxidation; the lack of difference between AP and Control supports absence of a pro-oxidant effect under basal conditions.

Effects of Dietary Apple Pomace and LPS Challenge on Splenic Protein Oxidation

Splenic protein oxidation, assessed by protein carbonyl content, was significantly affected by *E. coli*-LPS challenge and modulated by dietary apple pomace inclusion (Figure 3). Compared with the Control group, LPS challenge significantly increased protein carbonyl levels by 18.29% ($p < 0.0001$), indicating enhanced

protein oxidative damage under inflammatory conditions.

The diet with apple pomace prevented the increase in protein oxidation induced by LPS. In the AP+LPS group, protein carbonyl level was reduced by 17.27% relative to the LPS group ($p < 0.0001$). Moreover, protein carbonyl values in the AP+LPS group did not differ significantly from those in the Control group ($p = 0.0959$) or the AP group ($p = 0.8066$), indicating normalization of this oxidative stress marker.

Apple pomace alone (AP group) did not significantly affect protein carbonyl content compared with the Control group ($p = 0.4409$), with values being 1.32% lower than in Controls.

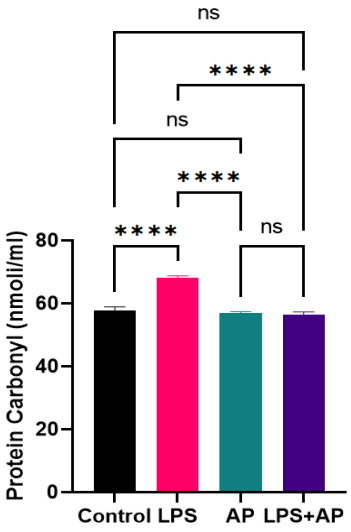

Figure 3. Effects of dietary apple pomace and LPS challenge on splenic Protein carbonyl marker in piglets after weaning. They were fed for 21 days with a Control diet (Control and LPS groups) or AP diet (AP and LPS+AP groups). On day 20, piglets from LPS and LPS+AP groups were challenged with 80µg LPS /b.w. The results are displayed as mean ± SEM. *= indicates a significant difference ($p < 0.05$); "ns" indicates no significance ($p > 0.05$)

Protein carbonyls are established markers of oxidative protein damage. LPS significantly increased splenic protein carbonyls, indicating that endotoxin-induced oxidative stress affected protein integrity in addition to lipids, consistent with inflammatory reactive oxygen species overproduction and with reports in LPS-challenged piglets showing increased oxidative damage alongside impaired antioxidant defenses (C. Li et al., 2020; Yin et al., 2014).

Apple pomace effectively prevented the LPS-induced increase, as protein carbonyl values in the AP+LPS group were reduced versus LPS and did not differ from Control or AP, suggesting stronger protection for protein oxidation than for some endpoints where recovery was partial. This is biologically meaningful because protein oxidation can impair enzyme activity, membrane transport, and immune cell function, which are relevant for splenic immune competence during post-weaning stress. The finding aligns with evidence that polyphenol-rich interventions reduce macromolecular oxidation during inflammatory challenge, despite marker- and tissue-dependent responses (Chedea et al., 2019; Huang et al., 2022).

The reduction in protein carbonyls likely reflects decreased reactive oxygen species availability through direct scavenging and improved enzymatic antioxidant protection, consistent with concurrent preservation of SOD and GPx, while the lack of effect in the AP group supports a challenge-dependent mechanism.

Effects of Dietary LPS Challenge on Splenic DNA Oxidative Damage

Splenic DNA oxidative damage, assessed by 8-hydroxy-2'-deoxyguanosine (8-OHdG) level, was significantly affected by *E. coli*-LPS challenge and modulated by dietary apple pomace (Figure 4). Compared with the Control group, LPS administration significantly increased 8-OHdG level by 61.76% ($p < 0.0001$), indicating marked DNA oxidative damage under inflammatory oxidative stress conditions.

The diet included apple pomace significantly attenuated the LPS-induced DNA oxidative damage. In the AP+LPS group, 8-OHdG level was reduced by 25.10% relative to the LPS group ($p < 0.0001$). However, 8-OHdG value in the AP+LPS group remained significantly higher than those in both the Control group ($p = 0.0243$) and the AP group ($p = 0.0019$), indicating partial protection of apple pomace against LPS-induced DNA oxidation. Apple pomace alone (AP group) did not significantly affect 8-OHdG level compared with the Control group ($p = 0.6010$), with values being 8.52% lower than in Controls.

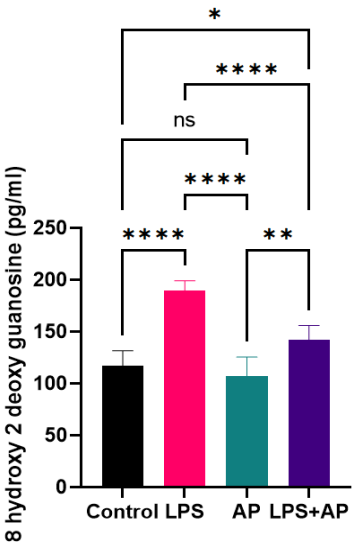

Figure 4. Effects of dietary apple pomace and LPS challenge on splenic DNA oxidation in piglets after weaning. They were fed for 21 days with a Control diet (Control and LPS groups) or AP diet (AP and LPS+AP groups). On day 20, piglets from LPS and LPS+AP groups were challenged with 80µg LPS /b.w.
The results are displayed as mean ± SEM. *= indicates a significant difference ($p < 0.05$); "ns" indicates no significance ($p > 0.05$)

8-OHdG is a marker of oxidative DNA damage. The increase in splenic 8-OHdG after LPS challenge indicates that oxidative injury extended to nucleic acids. A broader pattern reported in LPS-challenged piglets, showed that inflammatory challenge elevates oxidative damage markers together with impaired antioxidant defenses (Cui et al., 2024; Novais et al., 2020). Apple pomace significantly reduced 8-OHdG in LPS-challenged piglets, but values remained above Control and AP, indicating partial protection and matching the partial recovery pattern observed for TBARS and antioxidant enzymes. This is compatible with evidence that polyphenol-rich feeds limit oxidative damage in challenged animals, with efficacy influenced by tissue, marker, and challenge severity (Chedea et al., 2019; Cui et al., 2024). In this context, reducing splenic 8-OHdG is relevant because DNA oxidative damage can affect immune cell viability and function. The reduction likely reflects combined reactive oxygen species scavenging and preservation of antioxidant defenses, consistent with improved SOD and GPx, while the lack of difference between AP and Control indicates that apple pomace did not disturb basal DNA redox status.

Effects of Dietary Apple Pomace and LPS Challenge on Splenic Total Antioxidant Capacity

Splenic total antioxidant capacity (TAC) was significantly affected by *E. coli*-LPS challenge and modulated by dietary apple pomace inclusion (Figure 5). Compared with the Control group, LPS administration significantly reduced TAC by 2.29% ($p < 0.0001$), indicating a decrease in overall antioxidant defence capacity under inflammatory oxidative stress conditions.

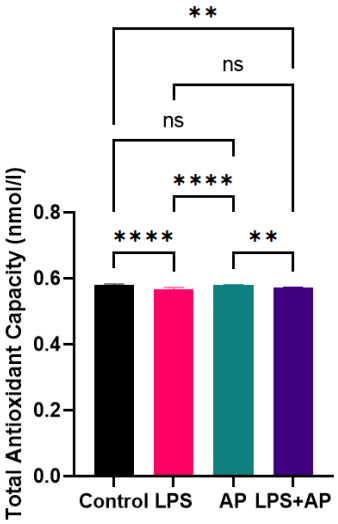

Figure 1. Effects of dietary apple pomace and LPS challenge on splenic TCA in piglets after weaning. They were fed for 21 days with a Control diet (Control and LPS groups) or AP diet (AP and LPS+AP groups). On day 20, piglets from LPS and LPS+AP groups were challenged with 80µg LPS /b.w.
The results are displayed as mean ± SEM. *= indicates a significant difference ($p < 0.05$); "ns" indicates no significance ($p > 0.05$)

The diet with apple pomace partially attenuated the LPS-induced reduction in TAC. In the AP+LPS group, TAC was 0.90% higher than in the LPS group; however, this increase did not reach statistical significance in the Tukey post hoc test ($p = 0.0931$). TAC values in the AP+LPS group remained significantly lower than those in both the Control group ($p = 0.0033$) and the AP group ($p = 0.0069$), indicating incomplete recovery of total antioxidant

capacity. Apple pomace alone (AP group) did not significantly affect TAC compared with the Control group ($p = 0.9982$), with values differing by only 0.12%. TAC provides an integrated estimate of antioxidant defense, reflecting combined enzymatic and non-enzymatic contributions. LPS significantly reduced splenic TAC, indicating reduced global antioxidant buffering capacity, consistent with reports linking decreased TAC with increased oxidative damage and depressed antioxidant enzymes in LPS challenge models (Jerez-Bogota et al., 2025; Jerez-Bogota et al., 2023).

In contrast to clearer effects observed for SOD, GPx, TBARS, and protein carbonyls, apple pomace produced only a modest, non-significant increase in TAC in LPS-challenged piglets, and TAC remained lower than in Control and AP. This suggests limited recovery of the total antioxidant pool under acute challenge conditions, which is plausible because TAC integrates multiple antioxidant systems and may be less responsive to partial enzyme recovery, particularly if low-molecular-weight antioxidants remain depleted after LPS exposure.

This endpoint-specific responsiveness aligns with the literature, where several interventions increase TAC in challenged piglets but responses vary by matrix, tissue, timing, and intervention type (Y. Li et al., 2020; Yan et al., 2024). Evidence from apple-derived interventions and fruit pomace studies supports antioxidant benefits in piglets, although direct spleen-specific TAC responses under LPS challenge remain limited (Ao et al., 2022; Jerez-Bogota et al., 2025). The non-significant TAC recovery may reflect the severity and timing of the acute LPS challenge, where ongoing oxidative demand can continue to deplete non-enzymatic antioxidants despite partial preservation of enzymatic defenses. Overall, the TAC data support a protective but limited effect of apple pomace on global splenic antioxidant capacity, consistent with partial recovery rather than complete normalization under acute inflammatory oxidative stress.

CONCLUSIONS

Taken together, the results obtained herein support a coherent interpretation, LPS impaired splenic antioxidant defense and increased oxidative damage to lipids, proteins, and DNA, while dietary apple pomace (at 5% inclusion rate) mitigated these effects to different extents depending on the endpoint. The strongest protection was observed for protein oxidation, partial protection for antioxidant enzymes, TBARS, and 8-OHdG, and only limited recovery for TAC, indicating a marker-specific protective profile under acute challenge conditions. These findings support apple pomace as a promising functional feed ingredient and agri-food by-product for improving oxidative stress resilience in weaned piglets, while highlighting the need for further studies on dose, duration, and mechanisms of action.

Further studies should define optimal inclusion levels, feeding duration, and the molecular mechanisms involved.

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