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Grape by-products and their efficiency in alleviating the intestinal disorders in post-weaning piglets

Gina Cecilia Pistol[1]*, Daniela Eliza Marin[1], Valeria Cristina Bulgaru[1] and Ionelia Taranu[1]

*corresponding author: gina.pistol@ibna.ro

[1] Laboratory of Animal Biology, INCDBNA-IBNA, National Research - Development Institute for Animal Biology and Nutrition, Balotesti, Romania

ABSTRACT

The post-weaning period is very stressful for piglets, leading to a transitory inflammation, alterations in the intestinal barrier, oxidative stress and a higher susceptibility to intestinal diseases with economic losses. The finding of new nutritional alternatives with anti-inflammatory, anti-bacterial, anti-oxidant properties is a challenge in post-weaning piglet's nutrition. Of these strategies, those based on agro-industry wastes rich in bioactive compounds are promising, the increasing number of studies focusing on the use of these wastes as successful modulators of post-weaning – associated disturbances in piglets. This review describes the role of grape by-products in the modulation of inflammation, reinforcement of the intestinal barrier and their role as antioxidant factors. Also, their in-depth intracellular mechanisms of action related to their anti-inflammatory, antioxidant properties are described.

Keywords: grape by-products, post-weaning, pigs, intestine, inflammation, oxidative stress, signalling pathways.

INTRODUCTION

The use of antibiotics in feed to prevent pathogenic infections and most often as growth promoters in animal farm sector was banned in the E.U. in 2006, due to the increased bacterial resistance and to the accumulation of antibiotics residue in animal products. Commercial pigs' sector was negatively affected because of this regulation, antibiotics being used frequently to control the post-weaning diarrhea. It is well known that post-weaning period is one of the most stressful events in pig life during which the maternal separation, the

major factor of stress along with changes of environment and the poor development of the digestive, enzymatic and immune systems increase the stress during this period. Also, piglets are highly susceptible to immune disorders and intestinal inflammation (Lallès, 2007) immediately after weaning.

At the moment, the current challenge for this sector and especially for pig research is to find new strategies to cover the bacterial spectra of antibiotics, promote gastrointestinal health in pigs and minimize the risk of bacterial resistance against antibiotics, as part of the One Health strategy currently demanded by consumers. This has opened up many opportunities for animal nutrition research to develop alternative feeding solutions to counteract the negative effects of weaning and to reduce mortality in piglets (De Lange, 2010). In the last decade many sources of proteins and bioactive molecules have been explored worldwide. The agro-industrial by-products are low-cost source of bioactive compounds which have been investigated in different formula, but which still deserve further investigations. Many by-products resulted from processing of plants for food and non-food purposes rich in biological active compounds have been studied for their prebiotic effect. These by-products have a broader range of feeding values and nutritional properties, the efficient, inexpensive and environmentally rational utilization of agricultural by-products being important for higher profitability and minimal environmental impact. In the last years, many research have been carried out to establish the effects of these by-products as potential anti-inflammatory, antioxidant and anti-microbial factors, as well as their efficiency in terms of gastro-intestinal health and as enhancers of the immune response of pigs after weaning. Also, many of these studies were performed in different models of intestinal damage in post-weaning piglets (diquat-treated, DSS- and TNBS-induced colitis, mycotoxin contaminated diet). The studies aimed to analyse the effects of by-products on unchallenged post-weaning piglets are limited, even if the weaning disturbances in piglets are a continuous problem in pig sector. The current review aims to presents new insights on the use of grape by-products as modulators of intestinal inflammation in post-weaning piglets. The scientific literature published in the English language was collected from Web of Science, Scopus, Pubmed Central and Google Scholar sources. A total of 86 references, covering 2002-2022 period were selected, criterion of selection being papers reported the effects of bioactive compounds from grape wastes on the intestinal health in weaning piglets. A synthesis on these effects on integrity and functionality of intestinal epithelium, inflammation and oxidative stress, and their associated signalling pathways was included.

Grape by-products and their content in bioactive compounds

Grape (*Vitis vinifera*) is one of the worlds' s largest fruit crops with a global production of around 78 million tons in 2018 (OIV, 2019). Grape processing generates a large number of by-products that can be broadly classified as follows: solid by-products (leaves, stems, seeds, skins, and pulp), highly viscous by-products (lees), and low-viscosity by-products (wastewater) (Bekhit, 2016). In the current paper will be pointed put the composition and the effects of grape pomace and grape seed by-products as main grape processing wastes.

The main by-product of the winemaking industry is grape pomace (or grape marc), which is represented by the solid organic material that remains from the crushing, draining, and pressing processes Grape pomace (GP) includes the skins and the pulp, usually the seeds and, in some cases, the stems. It is considered to be the most abundant winemaking by-product—it is estimated that the amount of GP corresponds to 20–30% of the original grape weight (Kalli, 2018; Oliveira, 2014). In the last years GP was taken into account as a source of commercially valuable products, with many benefits on animal and human health (Lelario, 2018; Sirohi R, 2020). Overall, the composition of GP is complex; it contains dietary fiber (19%–38%), the main constituent being insoluble fiber like cellulose and hemi-celluloses (Sirohi R, 2020; Antonić B, 2020). Also, GP contains proteins (4%–14%) (Sirohi R, 2020; Antonić B, 2020), lipids (1 – 13%), sugars (fructose, glucose) and a variety of minerals (Sirohi R, 2020; Antonić B, 2020). Several studies conducted on GP showed that this by-product had high antioxidant activity (Chidambara, 2002; Peixoto, 2018; Lingua, 2016) associated with polyphenol content and composition (Chedea, 2018).

Polyphenols represents approx. 70% of the bioactive compounds found in fruits and by-products derived from fruit processing (Swallah, 2020; Lipiński, 2017). These compounds have well-documented benefits on health, exhibiting antioxidant, anti-inflammatory, anticarcinogenic and antibacterial activities (Shahidi, 2015; Rasouli, 2017; Abbas, 2017; Ambriz-Pérez, 2016).

Of grape by-products, GP is the richest in polyphenols, approximately 60-70% of polyphenols from grape remaining in the pomace after wine obtaining (Bordiga, 2019). Its composition in these biologically active compounds gave GP significant attention due to the polyphenol`s health-promoting effects (Meini, 2019; Bordiga, 2019). Also, its composition in polyphenols could be a key factor involved in the positive effects of grape by-products (including GP) in animals (Antoniolli, 2015; Chedea, 2018). Total polyhenolic content in different GP samples was reported in the range of 1115.00 – 7475.00 mg GAE/ 100g (Negro, 2003; Dranca, 2019; Iora, 2014). As polyphenols, GP contain mainly flavonoid (anthocyanins, flavonols and flavanols) and non-flavonoid compounds (phenolic acids like hydroxycinnamic and hydroxybenzoic acids

and stilbenes) (Llobera, 2008; Chedea, 2018), identified also in grapes and wine (Spigno, 2007). GP represent a potential source of catechins, epicatechin, procyanidins, resveratrol (Chakka, 2022). Literature data reported the variable composition of grape poamce in anthocyanins, proanthocyanidins (procyanidins and prodelphinidins), and flavan-3-ols (Negro, 2003; Andelkovic, 2015).

Another grape processing by-products is *grape seed oil meal* (GSM), obtained after oil extraction from grape seeds. Both grape seeds and grape seed oil meal have a high content of fiber (40% w/w), proteins (11% w/w), carbohydrates, and minerals (Ma, 2017; Troilo, 2021), but they are rich in bioactive compounds such as polyunsaturated fatty acids (PUFAs) and polyphenols (ZhiJing, 2016). Of these constituents, fatty acid composition is highest in grape seed by-products. The PUFAs content of grape seed by-products is ranged mainly of 67.82% to 75.66% (Kollathova, 2020). Of PUFAs, a higher content of linoleic acid (from 66.6% to 73.8%) and α-linoleic acid (from 9.03% to 18.73%) was reported in grape seed and grape seed wastes (Fernandes, 2013; Hussein, 2015). Also, grape seed wastes are rich in oleic acid, as a monounsaturated fatty acid (MUFA) (Kollathova, 2020). Grape stems and grapes seed wastes contains also high amount of saturated fatty acids (SFA), from 18.45 to 32.88%, mainly palmitic (from 10.68 to 19.62%) and stearic acids (from 3.52 to 5.75%) (Kollathova, 2020; Gulcu, 2019). PUFAs exhibit a lot of health benefits, having strong anti-oxidative and anti-inflammatory properties, providing protection from cancer, inflammatory and autoimmune diseases (Kapoor, 2021).

Another nutrient found in substantial levels in grape by-products is dietary fiber. Literature data reported a content in total dietary fiber of up to 70% in GP (Bassani, 2020; Chakka, 2022). These studies showed that GP had a variable content of 26%-78% of insoluble dietary fiber such as cellulose and hemicellulose, and of 9-11% of water-soluble dietary fiber, including β-glucans, pectins, etc. (Bassani, 2020; Chakka, 2022). GSM contains an important amount of dietary fibres, from 48% to 34% (Salama, 2007; Elkatry, 2022), most of them (79-80%) being insoluble fiber (cellulose, hemicellulose and lignin) (D'Eusanio, 2023). The fibers from grape by-products are fermented in the colon, producing short chain fatty acids under the action of gut microbiota (Yu, 2013; Xiong, 2022); these SCFAs are reported to have shown a variety of biological effects, such as anti-inflammatory, immunoregulatory, anticancer and hepatoprotective effects (Xiong, 2022).

Owing to their health benefits, grape by-products can be employed as a source of bioactive ingredients with potential use in food industry, with application in both human and animal nutrition (Andelkovic, 2015).

The effects of grape by-products on the intestinal inflammation in piglets after weaning

The gastrointestinal tract is a multifunctional system, with major roles in digestion, nutrient absorption, water and electrolyte balance; besides these functions, the gastrointestinal system has an immune component, playing an important role in the regulation of immune homeostasis as well as innate and adaptive immunity (Takiishi, 2017). At weaning, piglets are exposed to multiple stressors (separation from the sow, change of diet from liquid sow milk to solid feeds); all these factors lead to histological and functional changes in the intestine, causing intestinal damage, abnormal immune responses and intestinal inflammation (Lallès, 2007). The current need in weaning stress management is to find nutritional alternatives which could have the potential to contribute to the reduction of the amplitude of intestinal inflammation around weaning. Due to their composition in bioactive compounds such as polyphenols and PUFAs, grape wastes are valuable modulators of inflammatory responses.

The key drivers of intestinal inflammation and of associated intestinal damage are cytokines and chemokines. It was demonstrated that interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), IL-18, IL-1β, and IL-17 cytokines as well as IL-8, CCL1 (Chemokine (C-C motif) ligand 1), and CCL20 (C-C Motif Chemokine Ligand 20) chemokines are overexpressed in the inflamed intestine and have been involved as contributors to intestinal damage (Andrews, 2018; Pié, 2004). It was postulated that the dietary modulation of the intestinal cytokines and chemokines production will need to be considered in order to design the most effective nutritional strategy for the management of weaning – associated intestinal inflammation.

Studies on the effects of grape wastes inclusion in diet of post-weaning piglets showed that the results obtained until now are promising, these wastes rich in polyphenols, PUFAs and fiber had the potential to modulate the cytokines and chemokines genes and proteins expressions in intestinal tissues. As bioactive compounds with well-demonstrated anti-inflammatory effects, grape wastes polyphenols were described as promising alternatives in piglet's nutrition, even if they were used as total polyphenolic extracts or as individual phenolic compounds. There are few studies demonstrated that grape waste extracts rich in polyphenols acts at all levels of intestinal segments, modulating the inflammatory markers in duodenum, jejunum, ileum, colon and caecum. For example, the inclusion in piglets diet of 1% grape seed and grape marc meal phenolic extract (with a high concentration of malvidin 3-glucoside, the major anthocyanin) was able to lower the mRNA expression of ICAM-1, CCL-2, TNF-α, IL-8 in duodenal mucosa, with no effect on IL-6 and IL-1β gene expression in duodenum (Gessner, 2013). Also, Fiesel et al (Fiesel, 2014) fed the piglets with a diet including polyphenol-rich grape

wastes (diet with 1% GSGME-grape seed and grape marc extract). The GSGME extract contained 8.5% total polyphenols with an increased abundance of gallic acid, catechin, epigallocatechin-3-gallate, epigallocatechin, epicatechin-3- gallate, epicatechin, proanthocyanidins, and anthocyanins. This GSGME diet leaded to a downregulation of the mRNA abundances of IL-8 and ICAM-1 in duodenum, of IL-1β, IL-8, TNF and ICAM-1 in ileum and of IL-1β, IL-8, TNF and ICAM-1 in colon (Fiesel, 2014). In caecum samples collected from post-weaning piglets fed diet with 5% GP the mRNA expression of pro-inflammatory cytokines IL-1β, IL-8, IL-6 and TNF-α decreased significantly compared with the control group, with no effect on the IL-10 anti-inflammatory gene expression (Wang, 2020). Piglets fed with 30 or 90 mg/kg resveratrol-supplemented diets prevented the diquat-induced increase in mRNA expression of TNF-α, IL-1β and IL-6 inflammatory cytokines in the jejunal mucosa of piglets (Xun, 2021). By contrast, Chen et al (Chen, 2021) showed that dietary supplementation of 150 mg/kg and 300 mg/kg resveratrol (a natural polyphenol found in grape seed and grape skin) had no effects on mRNA expressions of proinflammatory cytokines TNF- α, IL-1 β and IL-6 in jejunum. These authors reported that the anti-inflammatory effect of resveratrol was attributed to its effect on IL-10 anti-inflammatory cytokine, dietary supplementation of 300 mg/kg resveratrol increased the IL-10 gene expression in jejunal mucosa of piglets (Chen, 2021). Also, this study was focused on the effects of pure resveratrol, which acted only at the level of a single cytokine (IL-10) (Chen, 2021). The grape wastes had a content of more than one type of polyphenol, and their complex effects could be attributed to their diversity in polyphenol composition (Gessner, 2013; Fiesel, 2014; Wang, 2020).

In the previous section was underlined that grape by-products contains, in addition to polyphenols, other compounds with important biological roles, such as PUFAs and fibre. Even if the role of PUFAs in modulation of inflammation is well described, the use of grape wastes rich in PUFAs in the management of post-weaning disturbances in piglets was not so well studied so far. There are few studies focused on the effects of grape by-products as source of dietary PUFAs in attenuation of the intestinal inflammatory processes in piglets after weaning. An 8% inclusion of whole grape seed meal (GSM, rich in PUFAs and fiber) in the diet leaded to a significant decrease of TNF-α and IL-1β protein concentration in duodenum of weaning piglets (Pistol, 2019); the same diet with 8% GSM reduced the gene expression for IL-1α, TNF-α, IL-12p40, IL-17A and IL-6 pro-inflammatory cytokines in colon, when compared to piglets fed control diet (Pistol, 2020). Another study using the same inclusion in the diet of grape seed meal (8% GSM) reported that GSM diet induced a reduction of duodenal IL-1β and TNF-α pro-inflammatory cytokines concentration and a slight decrease of IFN-γ, and IL-8 concentration in the colon (Taranu, 2019). Also, a diet containing 10% mixture of grapeseed,

flaxseed and sea buckthorn meals (in a ratio of 3:4:1) (CFS diet) improved the inflammatory response in the colon of piglets, reducing the IL-1β gene expression and the IL-1β and TNF-α protein concentration (Marin, 2022). These effects were attributed to the high concentration of omega-3 PUFAs in the CFS diet (Marin, 2022).

These anti-inflammatory effects were observed in the piglets fed different grape by-products, being attributed to their composition in bioactive compounds and/or to their whole composition, the combination of polyphenols, PUFAs and fiber from grape by-products being able to act by synergic mechanisms. Also, their anti-inflammatory properties were demonstrated not only in duodenum but also in ileum, jejunum, colon and caecum, indicating that the active components of the grape by-products were not completely absorbed or degraded in the superior part of the small intestine, being at least in part available and active in the inferior parts of the intestine.

The effects of grape by-products on the integrity and functionality of intestinal epithelium in post-weaning piglets

During weaning and in post-weaning period, in the gut occur many morphological and functional changes which induce an impairment of the intestinal epithelial barrier, leading to the increase of the intestinal permeability (Lallès, 2007). This increase in permeability is accompanied by villi modifications (decrease of villi height) and reduction of the intestinal crypt epithelial cells proliferation (Tsukahara, 2012). The alterations in the intestinal wall morphology further destroys intestinal mucosal barrier function and impairs the digestive and absorptive capacity (Tang, 2022). Also, is affected the intestinal mechanical barrier, formed by epithelial cells joined by tight junction proteins, which consist of the transmembrane protein (e.g., claudins and occludins) and the cytosolic proteins (e.g., junctional adhesion molecule, Zonula-1, -2 and -3) (González-Mariscal, 2003). The expression levels of these junction proteins were strongly associated with the gut barrier function. The alteration of epithelial barrier function is related to a high production of pro-inflammatory cytokines, while this overexpression of pro-inflammatory cytokines during inflammation was associated with the impairment of intestinal barrier function (Liu, 2015). Cytokines such as TNF-α, IL-1β , Il-6 and IFN-γ are involved in the regulation of tight junctions, by decreasing the occludin expression (Mankertz, 2000), affecting the organisation of tight junction proteins, and impairing the barrier function (Zolotarevsky, 2002). The morphological elements of the intestinal barrier that are the most affected by weaning in piglets are: villi and crypts height, enterocyte proliferation and epithelial junction proteins (Modina, 2019). There are several polyphenol sources (e.g., extracts from oregano, ginger,

pepper, basil, cinnamon, garlic etc.) that have positive effects on the intestinal epithelium in in vitro as well as in vivo models (Modina, 2019; Sandoval-Ramírez, 2021). Regarding grape by-products, the studies on the effects of these wastes on intestinal barrier are focused on the morphological aspects (villi and crypt morphology and size) with less information on the modulation of junction proteins, mucus barrier and other elements involved in epithelial integrity. For example, Fiesel et al (Fiesel, 2014) found that in piglets fed with polyphenol-rich grape seed and grape marc meal extract was no evidence that this diet supplementation had effects on the villus height, crypt depth and their ratio in both the duodenum and jejunum. In another study, was reported that polyphenol rich red-wine pomace diet (3.3% red wine pomace inclusion in the diet) has an inhibitory effect on the jejunum villi growth in post-weaning piglets (Sehm, 2007). Also, the red-wine pomace diet increases the size of absorption area, by increasing the crypt area in the colon (Sehm, 2007). Gessner et al. (Gessner, 2013) observed that diet supplemented with grape seed and grape marc meal extract significantly increased villus height: crypt depth ratio in the duodenum of piglets. A grape seed proanthocyanidins (GSP) diet (250mg/kg) increased villus height of the jejunum and ileum while crypt depth significantly decreased compared with the control group; also, the ratio of villus height to crypt depth ratio in jejunum and ileum significantly increased in GSP fed piglets (Han, 2016). Similar results were reported by Rajkovic et al. which found that grape extract (GE) supplementation (150 g/t of feed) lead to an increase in villus height and villus surface area in jejunum and ileum collected from piglets (Rajković, 2022).

To date, a limited number of studies reported the effects of grape by-products and of their bioactive compounds on intestinal junction proteins expression in piglets after weaning. In a study of Cao et al. the supplementation of dietary resveratrol (100mg/kg) in piglets had no effect on the jejunal barrier function (measured by transepithelial electrical resistance and flux of fluorescein isothiocyanate dextran from the mucosa to the serosa). Also, the diet with resveratrol had no impact on the occludin, claudin-1 and ZO-1 protein expression in the jejunum of piglets (Cao, 2019). Han et al demonstrated that the dietary GSP increased the abundance of occludin protein in ileum and colon of piglets (Han, 2016). By contrast, the study of Chen et al. showed that chlorogenic acid, a natural polyphenol found in different plants including grape had protective effects on the intestinal barrier function in post-weaning piglets. Immunofluorescent analysis showed that in piglets fed chlorogenic acid (CGA) diet (1000 mg/g), claudin-1 was highly expressed and localized to the apical intercellular region of epithelium in the jejunum and ileum. Also, dietary CGA supplementation increased the ZO-1 and claudin-1 gene expression levels in ileum of pigs and tended to increase the expression of occludin and claudin-1 genes in the duodenal mucosa (Chen, 2018). Also, Marin et al. demonstrated that the CFS diet (containing 10%

mixture of grapeseed, flaxseed and sea buckthorn meals) rich in omega-3 PUFAs significantly increased the gene expression of occludin, claudin 7 and extracellular matrix protein ECM1 and of claudin 4 protein expression in the colon of piglets (Marin, 2022). By contrast, the inclusion of grape seed meal in the diet (8% GSM) did not affect the gene and protein expressions of tight junction proteins claudin-4, occludin and ZO-1 in the piglet's colon (Pistol, 2021). Even if these results are somewhat contradictory, the grape by-products have certain positive roles on the modulation of intestinal epithelium barrier integrity and function. These aspects need to be in-depth investigated in terms of the grape by-products capacity to influence a large palette of proteins involved in the villi and crypt junctions, as well as the intrinsic cellular mechanisms of control of these protein expressions.

The effects of grape by-products on oxidative stress in the gut of post-weaning piglets

Previous studies demonstrated that in piglets the weaning stress was closely related to oxidative stress. Generally, the oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defence (Pi, 2010). This imbalance can lead to the damage of DNA, proteins, and lipids, causing damage of the cellular structure and function (Pi, 2010). The significant increase of the markers of oxidative stress on the first day after weaning (protein hydroxyl, a marker of protein oxidative damage) and on the third day after weaning (blood malondialdehyde, MDA) indicated that an oxidative stress was induced in weaning piglets, with direct correlation with inflammatory processes and intestinal epithelium impairment described before (Yin, 2014). Also, weaning was associated with a decrease of copper- and zinc-containing superoxide dismutase (CuZnSOD), manganese-containing superoxide dismutase (MnSOD), glutathione peroxidase 1 (GPx1), and glutathione peroxidase 4 (GPx4) gene expressions in the small intestine of the piglets (jejunum), with significant decreases at day 3 post-weaning (Yin, 2014). When compared to suckling piglets, weaned pigs had a lower SOD2 and GPx1 gene expression level in the jejunum, a decreased activity of SOD antioxidant enzyme and a significant increased MDA and 8-OHdG (8-hydroxy-2´-deoxyguanosine, marker of DNA oxidative damage) concentration (Zhang, 2020). These enzymes belong to the complex system of antioxidant enzymes which protect the organism against the harmful prooxidants, by transforming ROS into more stable molecules and thus reducing the oxidative stress (Pi, 2010). The oxidative stress induced damages to the intestinal tissue of pigs, leading to bacterial translocation and compromising the intestinal barrier functionality (Yin, 2014).

The role of polyphenols in the modulation of oxidative stress was well documented, the phenolic compounds and flavonoids having strong anti-

oxidant properties, suppressing the ROS production by ROS scavenging, upregulation of antioxidant defence system or by inhibition of the enzymes involved in ROS formation (Hussain, 2016). Also, grape polyphenols improved the status of oxidative stress, the grape polyphenol supplementation increased the total antioxidant capacity, SOD, GPx and ORAC (Oxygen Radical Absorbance Capacity) levels (Sarkhosh-Khorasani, 2021). Some studies on the effects of grape by-products rich in polyphenol in weaned piglets confirmed the antioxidant properties of grape wastes due to their polyphenol content. For example, Chedea et al. demonstrated that a diet including 5% GP (GP) rich in polyphenols decreased the lipid peroxidation and increased the total antioxidant status in the duodenum and colon of piglets. Also, GP diet increased SOD activity in duodenum and CAT and GPx activities in the colon (Chedea, 2018). The authors postulated that this modulation of oxidative stress parameters was induced by the intestinal absorption of different polyphenols (procyanidin trimers and catechins) (Chedea, 2018). Similar results were reported by Hao et al. on piglets fed 50 mg grape seed procyanidins /kg feed (an increase in SOD, CAT and GPx activity and in the total antioxidant capacity with a reduction of lipid peroxidation in duodenum and colon) (Hao, 2015). Dietary GSPs (grape seed proanthocyanidins) supplementation (50, 100, or 150 mg/kg), leaded to an increase of SOD activity, of SOD1 and CAT gene expressions and reduced the MDA concentration in the jejunum, improving the intestinal antioxidant capacity in weaned piglets (Wei, 2022). Also, a study of Han et al (Han, 2016) demonstrated that dietary GSPs (250 mg/kg) increased the activities of SOD and GPx, whereas the content of MDA decreased in both serum and intestinal mucosal samples (equal amounts of jejunum, ileum and colon mucosa samples) of piglets after weaning. A diet supplemented with 1000 mg/kg chlorogenic acid increased the activities of GPx and CAT in jejunum and ileum, but did not affect the activity of SOD and the content of MDA in post-weaning piglets (Chen, 2018). Similar results were reported by Taranu et al (2019) which found that an inclusion of 8% grape seed meal in the diet enhanced GPx and SOD activities in duodenum and reduced lipid peroxidation (by measuring thiobarbituric acid reactive substances, TBARS) in duodenum, with no effects on these parameters in colon of weaned piglets (Taranu, 2019). The diets supplemented with either 300 mg/kg of resveratrol or with 300 mg/kg of pterostilbene (a dimethyl ether derivative of resveratrol) reduced the MDA concentration in the jejunum of weaning piglets; also, these diets leaded to an increase of GPx activity and of SOD2, GPx1 mRNAs levels in jejunum compared to piglets fed control diet (Zhang, 2020). By contrast, grape seed and grape marc diet did not influence TBARS concentration in plasma of piglets, decreasing the SOD1, GPx1 gene expression in the duodenal mucosa of piglets (Gessner, 2013). Similar results were found by (Rajković, 2022) in a study on grape extract (GE) supplementation in weaned piglets, with no effects on

TBARS concentration and also on the antioxidant enzymes (CAT, SOD and GPx) activities and gene expression in the intestinal tissues (jejunum and ileum). Collectively, these data suggested that bioactive compounds from dietary grape by-products may improve intestinal functions via enhanced antioxidant capacity.

The in-depth intracellular mechanism involved in the modulation of intestinal damage by grape by-products in weaned piglets

Recent studies have postulated that multiple signalling mechanisms are involved in weaning inflammatory and oxidative stress response, including mitogen-activated protein kinases (MAPKs), nuclear factor kappa B (NF-kB) and nuclear factor erythroid 2-related factor 2 (Nrf2) signalling pathways. Moreover, these pathways are cross-linked and are target of bioactive compounds from grape by-products.

The MAPKs are a family of serine threonine kinases which regulates cellular functions, such as differentiation, proliferation and cell death (Shifflett, 2014). The primary MAPKs classes are p38 MAPKs, ERKs and JNKs. Upon activation by phosphorylation, MAPKs enter into the cell nucleus, participating to the activation of the transcription factors, which results in the expression of target genes and a biological response (Shifflett, 2014). The prolonged activation of MAPKs induced the transcription and release of pro-inflammatory cytokines (IL-6, IL-8, TNF-α) leading to an inflammatory response (Xiao, 2020). Another key pathway whose activation can lead to pro-inflammatory cytokine production is NF-kB signalling. After activation by different factors (cytokines, ROS) the cytoplasmic NF-kB is translocated into the nucleus, activating the expression of a wide variety of genes, including those coding for mediators of inflammation (pro-inflammatory cytokines, chemokines and others) (Gessner, 2013). At weaning, those pathways are activated, contributing to the initiation and maintaining of inflammation at intestinal level. For example, the activated (phosphorylated) p38, total JNK and total ERK MAPKs are increased in the jejunal mucosa of weaning piglets (Hu, 2013) compared to pre-weaning piglets. Also, Yi et al (Yi, 2016) demonstrated that the activation of NF-kB is increased in the small intestine of weaning piglets. These signalling pathways could be targeted by bioactive compounds to reduce the amplitude of inflammatory processes around weaning. The literature studies have shown that administration of dietary sources of bioactive compounds (polyphenols and PUFAs) reduced the expression and/or activation of MAPKs and NF-kB in different in vitro and in vivo models of inflammation (Adkins, 2010) The modulation of the MAPKs and NF-kB signalling pathways by dietary sources of bioactive compounds in weaned piglets is still under debate, even of the results obtained until now are encouraging. Positive results were found by (Gessner, 2013), which

demonstrated that feeding of the weaned piglets with polyphenol-rich grape seed and grape marc meal extract reduces transactivation of NF-kB in the duodenal mucosa, lowering the transcript levels of NF-kB target genes involved in the inflammatory process. Also, Chen et al. (Chen, 2018) found that dietary chlorogenic acid supplementation decreased the mRNA expression levels of NF-kB in both duodenum and jejunum of piglets. Dietary resveratrol (300 mg/kg) administered to sows reduced the gene expression for 6 members of MAPKs signalling pathway in offspring at weaning (Meng, 2019), alleviating the weaning-associated jejunum inflammation. A diet with grape seed meal rich in PUFAs, polyphenols and fiber did not affect p38/ERK/JNK MAPK genes, except JNK1 gene in colon of piglets after weaning (Pistol, 2020); in the same study, no effect on NF-kB1 (NF-kB/p50) and RELA (NF-kB/p65) gene expressions was registered.

Another signalling pathway that is targeted by bioactive compounds, especially polyphenols is Nrf2 signalling, linked to the oxidative stress regulation by modulating the expression of antioxidant genes. Under normal conditions, Nrf2 binds to Keap1 in the cell cytoplasm, forming a Keap1-Nrf2 complex. The dissociation of Nrf2 from Keap1 occurs in case of activation by oxidative stress, and free Nrf2 is transferred into the nucleus, where activate the expression of downstream antioxidant enzymes (SOD1, SOD2, GPX1, and CAT) and phase II metabolic enzymes (NQO1 (NAD(P)H dehydrogenase (quinone) 1) and HO1 (hemoxygenase 1)), contributing to the maintaining of a normal redox state and to reduce the tissular damage (Gessner, 2013; Lu, 2014). In weaned piglets was registered a significantly lower NQO1 gene expression level in the jejunum, compared with the suckling piglets' diet (Zhang, 2020).

Several studies have shown that diverse polyphenols are able to activate Nrf2 signalling pathway, having a great antioxidative potential (Ahmed S.M., 2017 #43). However, less information is available about the effects of grape wastes or their bioactive compounds on the Nrf2 signalling in post-weaning piglets. The study of Zhang et al (2020) demonstrated that both resveratrol and its derivative pterostilbene included in the diet (300 mg/kg of feed) increased the gene expressions for Nrf2 substrates NQO1 and HO1 in jejunum compared to weaned piglets fed control diet (Zhang, 2020). These authors showed also that the diets with resveratrol and with pterostilbene increased the jejunal Nrf2 and NQO1 protein expression, with no effects on HO1 and Keap1 protein levels (Zhang, 2020). The attenuation of oxidative damage was more pronounced in case of piglets fed pterostilbene diet when compared to those received resveratrol diet, due to the increased reactivity and to the increased accumulation of nuclear NRF2 protein stimulated by pterostilbene. Also, in weaned piglets fed diet with 0.1% ellagic acid (a natural polyphenol found in a large variety of plants, including grape) the antioxidant capacity was improved in jejunum tissues, by increasing the mRNA levels of HO1

substrate of Nrf2 (Qin, 2022). Diet rich in polyphenols from grape seeds and GP did not increase but even reduced transactivation of Nrf2 and gene expression of several Nrf2 target genes (GPx1, SOD1 and NQO1) in duodenum of post-weaning piglets (Gessner, 2013). These authors assumed that the strong anti-oxidative properties of grape by-products extracts might suppress the local production of ROS, leading to the inhibition of Nrf2 signalling in the small intestine of piglets.

It was demonstrated a direct or indirect regulation between the members of Nrf2 and NF-kB signalling pathways, the knockdown of Nrf2 leading to an increase of NF-kB activation and of transcription of the NF-kB-dependent genes; also, an increased HO-1 expression inhibits NF-kB activity. The Nrf2 and NF-kB signalling pathways are cross-linked, controlling the transcription or function of target genes and respective proteins. NF-kB influences the Nrf2 signalling pathway by three main mechanisms: (1) the degradation of IKKβ (an inhibitor of NF-kB) by Nrf2 interacting protein Keap1, leading to the inhibition of the activity of NF-kB;(2) activation of Nrf2 by inflammatory mediators like COX2, inhibiting simultaneous the NF-kB activity; (3) the connection between the competitive Nrf2 transcriptional co-activator CBP and NF-kB (Ahmed, 2017). Also, both NF-kB and Nrf2 signalling pathways can be activated by upstream kinases including MAPKs, but the role of MAPKs in Nrf2 activation is still controversial (Surh, 2008). Due to their strong cross-linking, MAPKs, NF-kB and Nrf2 signalling pathways could be targeted by bioactive compounds from dietary grape wastes, leading finally to the positive modulation of intestinal damage, reduction of inflammatory processes and potentiation of antioxidant defence in piglets after weaning.

In conclusion, the grape wastes could be used as the dietary anti-inflammatory, antioxidants and epithelial defenders which could improve the intestinal damages occurred at weaning. These effects are modulated through inhibition of NF-kB and MAPKs signalling pathways, reduction of pro-inflammatory cytokines synthesis, activation of Nrf2 and scavenging ROS, alleviating epithelial disruption and the impairment of intestinal epithelium function in post-weaning piglets. Therefore, the fortification of cellular defence mechanism or restoration of stress-response signalling and attenuation of intestinal inflammation by intaking dietary grape wastes provides an important strategy for the management of weaning-associated disturbances in piglets.

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