THE EFFECT OF GRAPE WASTES, WINE INDUSTRY BY-PRODUCTS, ON INFLAMMATORY AND ANTIOXIDANT BIOMARKERS IN POST-WEANING PIGLETS

Gina Cecilia Pistol[1*], L.M. Palade[1], Daniela Eliza Marin[1], Mariana Stancu[1], Ionelia Taranu[1]

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

Abstract

Grape wastes, such as grape pomace and grape seeds are industrial wastes from the wine processing which is already known in scientific media, but does not yet have a particular interest in animal nutrition. These wastes are rich in bioactive compounds (polyphenols), with anti-inflammatory and antimicrobial properties. The aim of the present study was to investigate the effect of a compound feed containing 8% grape seed meal (GSM) on the humoral, systemic and local (duodenum) inflammatory response, and on oxidative stress parameters in weaning piglets. A total of 12 piglets, 6 piglets per group, were fed with 1) control diet or 2) 8% GSM diet for 30 days. At the end of feeding experiment, piglets were slaughtered. Concentration of immunoglobulin and pro-inflammatory cytokines and the activities of antioxidant enzymes, the total antioxidant capacity and the lipid peroxidation were assessed. Our results showed that GSM diet did not affect the humoral immune response, plasma concentration of IgA, IgM and IgG remaining unmodified. By contrast, at duodenum level, the GSM diet had anti-inflammatory effect, by reducing the concentrations of pro-inflammatory cytokines IL-6, IL-1β and TNF-α. GSM significantly increase the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in duodenum and reduced the level of lipid peroxidation. In conclusion, GSM by its content in bioactive compounds (polyphenols) and fibers is a valuable feeding alternative with positive effects in weaning and post-weaning period, with the capacity to reduce the inflammatory response in duodenum and the oxidative stress, by modulation of the antioxidant status at systemic and intestinal level.

Key words: weaning, piglets, inflammation, oxidative stress, polyphenols, by-products

INTRODUCTION

The processing of plants for food and non-food purposes generates a broad range of by-products, with a broader range of feeding values and nutritional properties than the processed raw materials. Efficient, inexpensive and environ- mentally rational utilization of agricultural by-products is of undisputed importance for higher profitability and minimal environmental impact. One of the higher value options is the recovery of bioactive plant food constituents, which could be used in pharmaceutical, cosmetics and food industry (Makris et al. 2007).

Grape wastes are wine industry by-products rich in bioactive compunds (Llobera and Cañellas, 2007). The investigations of chemical constituents of grape wastes (grape pomace and grape seeds) have shown the presence of antioxidant polyphenols, mainly anthocyanins, flavonols, flavonol glycosides, and phenolic acids (Makris et al., 2007).

Weaning is a major critical period of pig rearing because of increased susceptibility to gut disorders, infections and diarrhea. Weaning involves complex psychological, social, environmental and dietary stresses that interfere with gut development and adaptation (Lalles et al, 2007).

Gastrointestinal disturbances immediately post weaning cause large economic losses in the pig industry. Within the pig population total losses of all those born in the EU amount to approximately 17% and a substantial proportion of these losses can be associated with infections via mucosal surfaces (Lalles et

*Corresponding author: gina.pistol@ibna.ro
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al, 2007). From the nutritional perspective, controlling early intestinal inflammation is certainly a challenge in managing post-weaning gut disorders in piglets. The number of studies dealing with effects of by-products rich in polyphenols on the antioxidant status and inflammation in pigs as farm animal species is relatively limited. In a study of Gessner et al (2013), the diet containing grape seed and grape marc meal extract did not influence concentrations of plasma thiobarbituric acid-reactive substances (TBARS) and the antioxidative capacity of plasma and liver in piglets. Also, dietary grape wastes caused a downregulation of various pro-inflammatory genes (IL-1 β, IL-8, TNF-α, IL-6) and related signaling markers (Nrf2) in the duodenal mucosa of pigs (Gessner et al., 2013). It was demonstrated that polyphenol-rich grape wastes (grape seed and grape marc meal extract) could have anti-inflammatory effects (by reducing the mRNA levels for ICAM1, IL-1β, IL-8, CCL2 and TNF inflammatory markers) in various parts of intestine (duodenum, ileum, colon) (Fiesel et al., 2014). However, further investigations are needed before these substances can be considered for inclusion in commercial diets. Starting from these data, our study investigated the effect of a compound feed containing 8% grape seed meal (GSM) on the humoral, systemic and local (duodenum) inflammatory response, and on oxidative stress parameters in weaning piglets.

MATERIAL AND METHOD

Characterisation of grape seeds

Polyphenols extraction and determination of the total phenol content in grape seeds was performed as described in our previous studies (Chedea et al, 2017). Determination of the composition and concentration of polyphenols in grape seeds extracts by HPLC couple with mass spectroscopy (HPLC-DAD-MS) was performed as described in our previous study (Chedea et al, 2017).

Animals and experimental diets:

The feeding experiment was conducted in the experimental farm of IBNA on 12 weaned hybrid [(Landrace × Large White) × (Duroc × Pietrain)] piglets, with an average initial body weight of 10.80 ± 0.26kg, for 30 days. After one week accommodation, the piglets were assigned to two groups (6 piglets/group): 1) basal diet formulation (control CF) and 2) basal formulation with 8% grape seeds meal (CF- GSM). The basal formulation had 67.7% corn, 19% soybean meal, 4% corn gluten, 5% powder milk, 0.3% L-lysine, 0.1% methionine, 0.1% coline, 1.46% calcium carbonate, 1.37% monocalcium phosphate, 0.1% salt, 1% mineral-vitamin premix. CF-GSM formulation had a similar composition with the control formulation, with the difference of 58.5% corn, 18% soybean meal, 0.4% L-lysine, 0.15% methionine, 1.33% calcium carbonate, 1.42% monocalcium phosphate and 8% grape seeds meal.

The animals were housed in separate pens for each treatment, which allowed the exact measurement of the amount of ingested feeds and of the leftovers for each treatment. The animals had free access to the feeds and water. The animals were slaughtered in the end of the trial, according to the recommendations of the Ethics Committee of IBNA and to the European regulations; blood and organ samples were collected to evaluate the effect of CF-GSM formulation on anti-inflammatory and antioxidant parameters in plasma and duodenum samples.

Analysis of humoral immune response

The plasma concentration of immunoglobulin subclasses (IgA, IgM, IgG) selected as markers for humoral immune response and involved in the inflammatory response were measured using ELISA technique. Plasma samples were diluted according to manufacturer`s instructions (1:10,000 for IgA, 1:120,000 for IgG and 1:10,000 for IgM determination). The absorbance at 450nm was measured using a microplate reader (Tecan Infinite M200 PRO).

Analysis of the activities of antioxidant enzymes in tissue samples (duodenum)

For measurement of the antioxidant enzyme activity, specific enzymes' Cayman kit were used. 1g of frozen duodenum samples were homogenized in chilled phosphate buffer according to the manufaturer`s instructions and centrifuged at 1,500 or 15,000g for 15 min at 4°C. The obtained supernatants were used for assessment of the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). The absorbance

was measured using a Tecan microplate reader (Tecan Infinite M200 PRO).

Detemination of tisular lipid peroxidation (MDA- TBARS assay)

The level of lipid peroxidation in duodenum tissue was evaluated by measuring thiobarbituric acid- reactive substances (TBARS); 0.2g of duodenum samples were homogenized with 8 ml phosphate buffer, incubated at 95°C for 15 min and then cooled. The TBARS adducts were collected and the fluorescence was measured at 515 nm excitation and 548 emission with a Tecan microplate reader (Tecan Infinite M200 PRO). The results are expressed as nmol/g tissues

Analysis of the tissular parameters of the local inflammatory response

The tissular concentrations of the pro- and anti-inflammatory markers (IL-1 β, IL-8, TNF-α, IL-6) were measured by ELISA. The collected organ samples (duodenum) were lysed in saline phosphate buffer PBS with 1% IGEPAL, 0.5% sodium deoxycholate, 0.1% SDS and a cocktail of protease inhibitors. The samples were lysed for 30 minutes on ice, and centrifuged twice at 10,000 × g at 4°C for 10 minutes. The cytokines concentrations were determined by ELISA, using commercial kits (R&D Systems, Minneapolis, USA, and Biosource International, Camarillo, USA) according to manufacturers' instructions. Absorbance was measured at 450 nm using a microplate reader (Tecan Infinite M200 PRO).

RESULTS AND DISCUSSIONS

Polyphenols content of grape seeds extract

The grape seeds extract contain a large amount of total polyphenols, 5567.22 mg GAE/100 g sample. HPLC-MS analysis showed that GS extract contain favonoids (catehins, epicatechins, procyanidins), the highest concentration being observed for caffeoylquinic acid (57.36mg/100g), cerulic acid derivate (34.43mg/100g) and for dicaffeoylquinic acid (28.85mg/100g).

CF-GSM effect on the humoral immune response

The humoral response mediators are immunoglobulins, formed in spleen and lymph nodes and secreted by mature plasma cells.

In our study, the effect of dietary GSM on the humoral immune response was assessed by the measurement of the concentrations of immunoglobulin subsets, IgA, IgM, and IgG in plasma collected from piglets at the end of feeding experiment, and the results were shown in Figure 1.

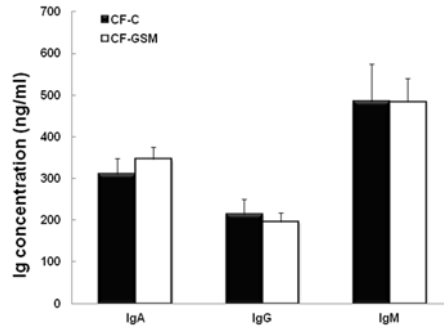

Fig. 1. Plasma immunoglobulins (IgA, IgG, IgM) concentration in weaning piglets fed diet including 8% GSM

As shown in Figure 1, the presence of GSM in the diet of piglets did not have any influence on plasma IgA, IgG and IgM compared with piglets receiving control diet. The effects of polyphenols on humoral immune response seemed to be dependent on the duration of the treatment. In very recent study, Fu et al (2018) showed that in the first week of 14-days trial on piglets feeding different doses of resveratrol powder (0.1g/kg bw; 0.33g/kg bw; 1.0g/kg bw), the levels of IgG, IgM, and IgA in serum were increased in varying degrees with different dose of resveratrol supplementation, while these effects could not be observed at the end of the second week.

CF-GSM effect on the concentration of pro-inflammatory cytokines in duodenum samples

In weaning and post-weaning period, the duodenum is a very sensible organ to inflammation. To assess the effect of the GSM diet on inflammatory response at this level, we measured concentrations of pro-inflammatory cytokines TNF-α, IL-6, IL-1β and IL-8. Our results presented in Table 1. Our results showed that GSM diet leaded to a

significant decrease of TNF-α (-46%, p=0.008), IL-1β (-47%, p=0.010) and IL-6(-14%, p>0.005) in duodenum samples collected from piglets. The modulation of cytokine concentration and/or gene expression by plant polyphenols was studies, and the results showed that these compounds could reduce the level of pro-inflammatory cytokines. It was demonstrated that diets including polyphenol-rich plant wastes (1% grape seed and grape marc meal extract inclusion in piglets diet, Gessner et al, 2013; diet with 1% GSGME-grape seed and grape marc meal extract, with a total polyphenols content of 5%, Fiesel et al., 2014) were able to lower the expression of TNF-α, IL-6, IL-1β and IL-8 proinflammatory genes in duodenum and in other intestinal tissues (ileum and colon). Jang et al. (2016) reported that feeding different doses of cocoa powder (2.5, 10 and 20 g) containing 51, 205 and 410 mg flavanols, respectively, to pigs for 4 weeks decreased gene expression of TNF-α and Toll-like receptors -2, -4 and -9 in the ileal Peyer`s patches, mesenteric lymph nodes and the proximal colon.

Table 1 The effect of diet containing 8% GSM on cytokine concentration in duodenum samples collected from weaning piglets

Cytokine	Experimental group		*p*-value (CF-GSM *vs* CF-C)
	CF-C	CF-GSM	
TNF-α (ng/ml)	119.1±23.3	63.7±11.3	<0.01
IL-1β (ng/ml)	1277.8±273.8	672.1±89.7	<0.05
IL-8 (ng/ml)	6808.2± 316.3	7094.1±344.6	NS
IL-6 (ng/ml)	779.1±252.6	671.6±120.9	NS

CF-GSM effect on the activity of antioxidant enzymes and lipid peroxidation in duodenum samples

The analysis of the effect of 8% GSM diet on the activities of main antioxidant enzymes (CAT, SOD and GPx) showed that CF-GSM significantly increase the activity of SOD (+4%, p = 0.008) and GPx (+20%, p = 0.020) in duodenum (Table 2). Also, 8% GSM in diet leaded to a decrease of lipid peroxidation in duodenum (-51%, p<0.001), as shown in Figure 2.A. No effect on antioxidant status was found. The study of Gessner et al (2013) reported that the diet containing 1% mixture of grape seed and grape marc meal did not affect the concentrations of α-tocopherol, TBARS and antioxidative capacity in piglet plasma and liver. Similar with our results on 8% GSM diet, Chedea et al (2018) demonstrated in an *in vivo* study that a 5% grape pomace in piglet's diet increased the total antioxidant status and decreased lipid peroxidantion (TBARS) in both duodenum and colon, and increased SOD activity in duodenum and CAT and GPx activity in colon. In the study of Ebrahimzadeh et al. (2018), the inclusion in chicken diet of grape pomace (5%, 7.5% and 10%) differentially modulate the antioxidant status and the activity of antioxidant enzymes, in relationship with concentration of grape pomace.

Table 2 The effect of CF-GSM on CAT, GPx and SOD enzyme activity in duodenum samples collected from weaning piglets

Experimental group	Enzyme activity		
	CAT (µmol/min/g tissue)	GPx (µmol/min/g tissue)	SOD (U/g tissue)
CF-C	3.6±0.1	2.1±0.03	453.9±3.5
CF-GSM	3.5 ±0.2	2.6±0.1	471.3±3.9
***p*-value (CF-GSM *vs* CF-C)**	NS	<0.05	< 0.01

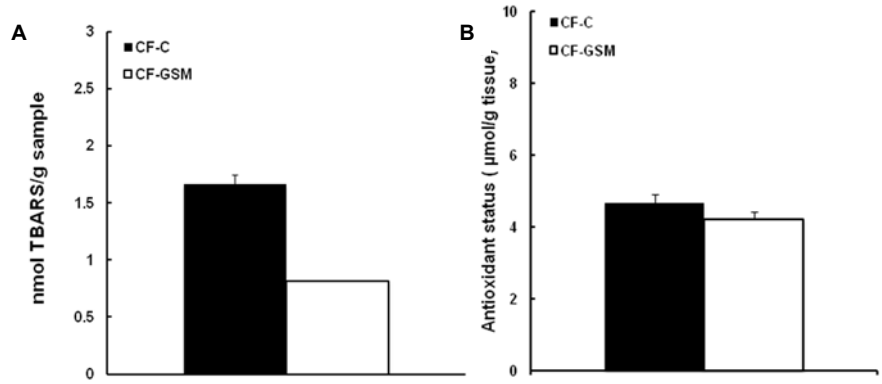

Fig. 2 The effect of 8% GSM inclusion in diet on lipid peroxidation (A) and antioxidant status (B) in duodenum samples collected from weaning piglets

CONCLUSIONS

In conclusion, the present study shows that inclusion in diet of 8% polyphenol rich GSM had anti-inflammatory effects at duodenum level and thus might provide a useful dietary strategy to inhibit inflammation in the gut, frequently occurring in pigs, particularly after weaning. Also, feeding GSM increases the activity of antioxidant enzymes and reduced the level of lipid peroxidation in piglet duodenum. Dietary GSM could be useful feed supplements in weaning and post-weaning piglet nutrition, in order to improve both animal health and anti-inflammatory and antioxidant status.

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