Assessment of the ability of dietary yeast-fermented rapeseed meal to modulate inflammatory and oxidative stress in piglets after weaning

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ABSTRACT. The aim of the study was to investigate the potential of a diet containing rapeseed meal fermented by *Saccharomyces cerevisiae* as a new sustainable feed to reduce transient intestinal inflammation, diarrhoea and oxidative stress in piglets after weaning. In this study, 16 male post-weaning piglets, with an initial weight of 9.04 ± 0.19 kg, were randomly allocated to two dietary treatments: control and 10% fermented rapeseed meal (FRSM) – 8 pigs/treatment. The experiment lasted 21 days. At the end of the trial, the animals were slaughtered and samples of blood and segments of the jejunum, ileum and colon were collected for determination of plasma biochemical, inflammatory and oxidative stress parameters. Pig performance and diarrhoea incidence were also investigated. The results showed that the FRSM diet had no significant effect on piglet performance, weight and average daily weight gain, as well as plasma biochemical parameters. However, the number of piglets with diarrhoea was higher in the control group than in the group receiving the FRSM diet throughout the experimental period. Moreover, a decrease in TNF-α ($P = 0.03$) and IL-1ß ($P < 0.05$) cytokine levels was recorded in the colon and jejunum samples from the FRSM group. In addition, IL-8 and IL-6 concentrations were decreased ($P = 0.0009$ and $P = 0.03$, respectively) in the ileum of piglets fed FRSM, indicating the modulatory capacity of this feed in reducing weaning-associated intestinal inflammation. The FRSM diet also improved the antioxidant status and significantly reduced lipid peroxidation and thiobarbituric acid reactive substance levels in plasma ($P = 0.022$) and in the jejunum ($P = 0.028$) and colon ($P = 0.003$), suggesting the potential of fermented rapeseed meal to limit oxidative reactions. In conclusion, the present study showed that fermentation of rapeseed meal using *S. cerevisiae* enriched the nutrient composition and reduced the concentration of anti-nutrients (e.g. glucosinolates). Moreover, the addition of FRSM to diets of pigs after weaning improved their intestinal health status, indicating its beneficial effect.

Introduction

The ban on the use of antibiotics in feed (EC, 2006) has had a significant impact on the livestock sector, especially for pigs after weaning (Taranu et al., 2015). Consequently, it is necessary to find new sources of antimicrobial compounds, which has opened up many opportunities for animal nutrition research. The processing of plants (oilseeds, cereals, medicinal plants, etc.) for feed or food, or non-food products (e.g. oil, alcohol, kerosene, etc.) generates a wide range of by-products with significant

nutritional values and bioactive properties that can be an important and applicable alternative to conventional feed (Dragomir et al., 2015). These by-products can also be easily processed by modern biotechnologies that increase their nutritional and energy value, while reducing or removing undesirable anti-nutritional factors. The processing and utilisation of by-products is also very important for the environment. Fermented by-products have been recently of great interest as fermentation is considered an efficient way to increase feed nutritional quality, thereby improving animal performance, gut immunity and its bacterial ecology (Hong et al., 2004; Zhu et al., 2017). Microbial fermentation is very efficient in eliminating anti-nutritional factors and enriching the diet in bioactive components such as vitamins, enzymes, organic acids, etc. (Czech et al., 2021). For example, the effect of *Aspergillus oryzae* GB-107 fermentation on the nutritional quality of food-grade soybean and feed-grade soybean meals showed that the fermentation increased protein content by 10%, while eliminating trypsin inhibitors and reducing peptide size (Hong et al., 2004). Furthermore, Lee et al. (2007) reported a 3–18 times higher free amino acid content and a higher protein digestibility compared to control in soybean meal after two-stage fermentation by lactic bacteria and mould. Fermented feed has been studied in farm animals such as poultry (broilers, ducks), but piglets were of particular interest because they are very sensitive after weaning and demonstrate reduced performance, diarrhoea, inflammation and higher mortality (Lallès et al., 2004; Satessa et al., 2020b). The use of soybean meal fermented by a probiotic mixture (*Lactobacillus plantarum*, *Bacillus subtilis* and *Saccharomyces cerevisiae*) in the diet of post-weaning piglets reduced diarrhoea caused by *E. coli* and improved intestinal morphology and digestive enzyme activity (Yuan et al., 2017; Zhu et al., 2017). Studies of Yuan et al. (2017) and Feng et al. (2020) also showed that the addition of fermented soybean meal to the diet of weaned piglets positively affected nutrient digestibility, intestinal enzyme activity, faecal microbiome and performance. Although soybean meal has many advantages, it remains an expensive feed raw material, dependent on import, with rapid changes in its availability (Onarman Umu et al., 2018). Crisis situations, such as the Covid19 pandemic, have shown that downtimes may occur in its supply (Chuang et al., 2021), and thus local alternatives needs to be found. Rapeseed meal,

a by-product of the oil industry, is a valuable feed ingredient rich in high-quality proteins, unsaturated lipids, fibre and phytochemicals (e.g. polyphenols, organic acids, vitamin E, vitamin B complex and mineral elements) (Mejicanos et al., 2016; Li et al., 2020). It has been used in pig nutrition as an alternative to soybean meal, but in low concentrations due to the negative effects caused by the presence of anti-nutritional factors such as glucosylates (Fazhi et al., 2011). Rapeseed fermentation has been proposed as a viable solution to reduce glucosynolate contents, increase palatability and improve digestibility of crude protein and phosphorus. It has also been claimed that fermentation improves fibre digestion, reduces phytic acid concentration, while increasing lactic acid levels (Grela et al., 2019). To date, several types of fermentation have been applied in rapeseed meal, e.g. lactic fermentation (*Lactobacillus fermentum*, *Bacillus subtilis*) or fungal fermentation (*Aspergillus sp.*). These microorganisms responsible for the fermentation process are a source of phytase that destroys phytase complexes, resulting in a decrease in antinutritional factors like phytic acid (Wang et al., 2010b). Nutrition studies with fermented rapeseed meal have focused particularly on growth performance, energy and nutrient digestibility (Chen et al., 2018). Thus, the inclusion of *Lactobacillus*-prefermented rapeseed meal in broiler chicken diets was shown to enhance feed digestibility and utilisation, gut morphology and antioxidant capacity based on the reduction of anti-nutritional factors (Hu et al., 2016). Replacing soybean meal with fermented rapeseed meal cake stimulated the antioxidant status by decreasing lipid hydroperoxides (LOOH) and malondialdehyde (MDA), while increasing antioxidant enzyme (superoxide dismutase – SOD, glutathyon peroxidase – GPx) levels in turkey (Drażbo et al., 2018). Feeding weaned piglets with increasing dietary proportion (8, 10, 12, 15 and 25%) of rapeseed meal prefermented by lactic acid bacteria improved growth performance, increased some haematological parameters and intestinal histomorphometric indices, as well as elevated immunoglobulin (IgA, IgM and IgG) and interleukin-6 titres; the optimum effect was observed for lower doses of prefermented meal (8 and 10%) (Satessa et al., 2020b). In another study, these authors reported that lactic acid bacteria-fermented rapeseed meal added to the diet (10%) was able to stimulate better the development of jejunum villi, colon mucosa and colonic microbiota composition than

the control feed and feed with ZnO supplementation used as positive control (Satessa et al., 2020a). Data on the effect of fermented rapeseed meal on immune response, inflammation and oxidative stress, i.e. processes that are significantly affected in pigs during the weaning period are still limited. In addition, according to our knowledge, the use of yeast-fermented rapeseed meal has not yet been investigated in pigs. Therefore, the current study was carried out to assess growth performance, diarrhoea incidence, immune response parameters, inflammatory and antioxidative status in piglets fed a diet with 10% rapeseed meal fermented by *Saccharomyces cerevisiae*. The ability of in-feed fermented rapeseed meal to modulate markers of inflammation and oxidative stress signalling pathways was also investigated.

Material and methods

Fermentation of rapeseed meal

Rapeseed meal was fermented with *S. cerevisiae* (EC1118 – commercial winemaking yeast, Nizza Monferrato, Italy). Briefly, medium (Saburaud, Sigma, Saint Louis, MO, USA) for yeast fermentation was inoculated with *S. cerevisiae* extract (10 g/l) and fermented for 24 h at 37 °C with shaking at 130 rpm. Then the medium was centrifuged 4 min at 3 000 rpm. The supernatant was discarded and yeast was resuspended in 800 ml of distilled water. Rapeseed meal was placed in sterile plastic bags (1 kg/bag) (Multi Lab, Buchares, Romania), inoculated with resuspended yeast and fermented for 24 h. Prior to the *in vivo* experiment, a series of tests on the effect of fermentation time were carried out in the laboratory. Two time periods were tested: 24 and 72 h. The results did not show significant differences. After fermentation, the content of each bag was homogenized and mixed with 2.5 l of distilled water, allowed to decant, and then the supernatant was discarded. The contents of bags were transferred onto a sterile plastic plate (Roth Rotilabo, Karlsruhe, Germany) in a uniform layer and dried at 38 °C (Ecocell oven, BMT Medical Technology, Stadlern, Germany) until a moisture content of about 60–65%. After drying, the fermented rapeseed meal was packed in paper bags and stored in a cool and dry place until added to the compound feed (complete feed) together with other ingredients of the experimental diet. Both unfermented and fermented rapeseed meal were analysed for their main nutrient components before use (Table 1).

Table 1. Rapeseed meal composition

	Rapeseed meal	
	unfermented	fermented
Dry matter, %	91.99	89.71
Crude protein, %	33.51	35.08
Ether extract, %	1.23	0.85
Crude fibre, %	8.38	11.89
Ash, %	6.57	6.32
Ca, %	0.58	0.56
P, %	1.18	1.31
Na, %	0.009	0.01
K, %	1.44	1.05
Mg, %	0.58	0.54
Fe, ppm	208.03	266.38
Cu, ppm	25.15	25.95
Mn, ppm	174.86	226.42
Zn, ppm	495.91	536.52
Total polyphenols, mg GAE/g	88.94	54.13
Glucosinolates, mg sinigrin/g	4.78	2.39

GAE – gallic acid equivalent

Animals and diets

The experimental protocol was approved by the Ethics Committee (No. 118/2019) of the National Research-Development Institute for Animal Nutrition and Biology, Balotesti, Romania. Animals were cared for in accordance with the Romanian Law 43/2014 for handling and protection of animals used for experimental purposes and the EU Council Directive EC/63/2010 on the protection of farmed animals.

A total of 16, 27-day-old male weaned piglets (TOPIG hybrid [(Landrace × Large White) × (Duroc × Pietrain) NORVEGIAN], provided by a private breeder (Fermeplus, Cazanesti, Romania), were used in this study. Animal protocol was as follows: three days after birth, the piglets received liquid milk substitute in addition to sow's breast milk and at the age of one week (7 days), the milk replacer was removed and the piglets had access to liquid and solid pre-starter feed. The piglets were weaned at 27 days of age and average body weight of 9.06 ± 0.14 kg and randomly assigned, based on their body weight, to one of two experimental diets formulated to meet the NRC (2012) requirements for weaned pigs: 1) a starter diet based on corn-soybean meal as a control diet (control diet); 2) a modified control diet with soybean meal replaced with 10% fermented rapeseed meal as an experimental diet (FRSM diet) (Table 2). Both diets were prepared in a powder form the day before the start of the experimental trial, stored in a cool and dry place and daily administered to animals for 21 days after

weaning. During the experimental period, feed and water was administered *ad libitum.* The animals were individually ear-tagged and housed in pens. Eight pigs were used for each experimental group (two replicates per group and 4 pigs per replicate). Pigs were weighed (balance SWS-International, Bucharest, Romania) individually at the beginning and at the end of the experimental period, and daily weight gain was calculated from the obtained difference. Feed intake was measured weekly at the pen level. The occurrence of diarrhoea was checked daily for each piglet and the percentage in relation to the total number of piglets was calculated as described by Pistol et al. (2020).

Sample collection

At the end of the experimental period, blood samples were aseptically collected into 9-ml Vacutainer tubes containing 14.3 U/ml lithium heparin (Vacutest Kima, Arzergrande, Italy) from the jugular vein of all piglets before slaughter. Subsequently, they were centrifuged at 775 *g* for 25 min (SIGMA 2-16KL, Berlin, Germany) for plasma separation. All pigs were fasted, electrically stunned, exsanguinated and samples from the jejunum, ileum and colon were collected. Portions of the jejunum, ileum and colon were perfused with ice-cold saline solution (1:9, w/v) to remove blood and stored at −80 °C until analysed for inflammatory and anti-oxidant stress markers. Before analysis, gut aliquots were homogenized in liquid nitrogen to a fine powder using an analytical mill (IKA-Werke, M-20, Stouten, Germany).

Blood plasma biochemistry measurement

Blood plasma was assessed for general health parameters such as total cholesterol, total protein, urea, creatinine, hepatic markers (aspartate-aminotransferase – AST, alanine aminotransferase – ALT, gamma glutamyltransferase – GGT, alkaline phosphatase – ALP and bilirubin) and electrolytes (Ca, P and Mg) using an automated clinical chemistry analyser (Pentra C400, Horiba, *Montpellier,* France).

Measurement of feed chemical composition

The chemical composition of rapeseed meal and experimental feed: dry matter, crude protein, crude fat, crude fibre and ash was analysed from unfermented and fermented rapeseed meal and from complete feed in triplicate, according to the methods of the International Standard Organization (ISO, 2010). Concentration of mineral elements (Ca, P, Na, K, Mg, Fe, Cu, Mn, Zn) was measured using flame atomic absorption spectrophotometry with Zeeman background correction and a graphite furnace (Pye Unicam, Thermo Electron, Solaar M6, Cambridge, UK). Total polyphenols were determined by the Folin-Ciocalteu reaction and polyunsaturated fatty acids (PUFA) using gas chromatography (Clarus 500, Perkin Elmer, Shelton, CT, USA), as described by Untea et al. (2012) and Palade et al. (2019).

Glucosinolate levels were measured after water extraction using high-performance liquid chromatography with a diode array detection detector (Agilent Technologies 1200 Series, Morge, Switzerland), using a Phenomenex Luna Omega Polar C18 column (100 Å, 250 × 4.6 mm, 5 μm) at a temperature of 25 °C. Concentration was expressed as mg of sinigrin used as an external standard.

Detection of inflammatory and humoral immune response markers

Gut (jejunum, ileum and colon) samples frozen in liquid nitrogen (2 mg), prepared as described above, were homogenized in phosphate buffer containing 1% IGEPAL, 0.1% SDS, 0.5% sodium deoxycholate (Sigma-Aldrich, Saint Louis, MO, USA) and complete protease inhibitor cocktail tablets (EDTA free, Sigma-Aldrich, Saint Louis, MO, USA). The homogenates were kept on ice for 30 min and subsequently centrifuged twice at 10 000 *g* at 4 °C for 10 min. Inflammatory markers such as pro-inflammatory cytokines IL-1ß, IL-6, IL-8, TNF-α, IFN-γ were assayed by ELISA in undiluted supernatants using R&D kits (anti-swine primary and biotinylated secondary antibodies included; Minneapolis, MN, USA) according to the manufacturer's instructions (Marin et al., 2013). IgA, IgM and IgG were also determined in plasma using ELISA according to the manufacturer's instructions (Medist, Montgomery, TX, USA), as described by Taranu et al. (2018). A Tecan microplate reader (Tecan Infinite M200 PRO, Salzburg, Austria) was used to measure the absorbance at 450 nm. Protein recombinant cytokines (IL-1ß, IL-6, IL-8, TNF-α and IFN-γ) were diluted according to the manufacturer's instructions and used as standards to prepare the standard curve.

Detection of antioxidant marker activity

The activity of antioxidant markers such as SOD, catalase (CAT) and GPx was measured in plasma and intestinal segments (jejunum, ileum, colon) using Cayman's kits and instructions (Ann Arbor, MI, USA). Briefly, 1 ml of plasma and 0.2 g of frozen powdered tissue obtained by homogenization in liquid nitrogen was further homogenized in chilled phosphate buffer (50 mM potassium phosphate, containing 1 mM EDTA per gram of

tissue for CAT, 20 mM HEPES buffer for SOD and 50 mM Tris-HCl (pH 7.5, containing 5 mM EDTA and 1 mM DTT per gram of tissue for GPx) and centrifuged at 1 500 or 15 000 g at 4 °C for 15 min. The resulting supernatant was used to determine enzymatic activities. The absorbance was read at 540 nm (CAT), 440–460 nm (SOD) and 340 nm (GPx) using a microplate reader (Tecan Infinite M200, Salzburg, Austria).

Detection of oxidative stress and intestinal barrier integrity markers

Lipid peroxidation (thiobarbituric acid reactive substances – TBARS) and intestinal barrier integrity markers (diamine oxidase – DAO) were assayed in intestinal segments (jejunum, ileum, colon) and plasma using commercial kits and instructions supplied by Cayman Europe (Tallin, Estonia) and Sigma-Aldrich (St. Louis, MO, USA), respectively. Plasma samples were homogenized in deionized water, 0.5 N HCl and thiobarbituric acid (TBA), (Sigma-Aldrich, St. Louis, MO, USA). Intestine samples were homogenized in buffers provided in individual kits, centrifuged at 15 000 and 10 000 g for 15 or 5 min at 4 °C. The resulting supernatant was used to assess enzyme activity. TBARS and DAO fluorescence was measured at 548 nm with excitation at 515 nm (TBARS) and 535/587 nm in kinetic mode for DAO using a microplate reader (Tecan Infinite M200, Salzburg, Austria).

Immunoblot analysis

Immunoblot analysis was used to detect total protein expression of NF-κB (p65) and nuclear factor erythroid 2-related factor 2 (Nrf2) signalling molecules in intestinal segments (jejunum, ileum and colon) as described by Marin et al. (2020). Briefly, after separation using 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the samples were transferred onto 0.45 µm nitrocellulose membrane and 5% non-fat dry milk blockage overnight. Membranes were incubated with primary rabbit anti-porcine antibodies for β-actin*,* phospho-NF-κB/p65 and Nrf2 (Cell Signalling Technology, Beverly, MA, USA and Abbexa, Cambridge, UK, respectively) for two hours at room temperature. The membranes were subsequently incubated with secondary antibody (horseradish-peroxidase-conjugated anti-rabbit IgG, Cell Signaling Technology, Danvers, MA, USA) for one hour. Immunoblotting images were developed using a MicroChemi Imager (DNR Bio-Imaging Systems LTD, Neve Yamin, Israel) and protein expression level was evaluated using GelQuant software (DNR Bio-Imaging Systems LTD, Neve Yamin, Israel).

Statistical analysis

The results are presented as means ± standard error (SEM) from two independent determinations. Histograms created using GraphPad Prism 8.0 (GraphPad Inc. La Jolla, CA, USA) were used for graphical representation. Statistical differences between the two groups receiving different diets were assessed using one-way ANOVA and Student's t-test (SAS Analytics, USA), followed by Fisher's least square difference procedure. Each animal was considered an experimental unit for all determined parameters with the exception of feed consumption and the gain:feed ratio. P-value equal or lower than 0.5 was considered significant and P-value of 0.1 was considered a trend.

Results and discussion

Chemical composition of fermented versus unfermented rapeseed meal

Yeast fermentation processes caused several changes in the nutritional composition of rapeseed meal (Table 1). Yeast is a rich source of valuable molecules and fermentation of rapeseed meal by yeast contributed to increased levels of crude protein fibre and mineral elements (P, Na, Fe, Cu, Mn and Zn), with the highest concentration recorded for Fe (266.38 ppm vs 208.03 ppm) and Mn (226.42 ppm vs 174.86 ppm), two elements important in immune response, while a decrease in crude fat content was observed.

Glucosinolates are among the most toxic anti-nutritional compounds from rapeseed (Drażbo et al., 2020) that reduce animal performance, as well as affect immune response and internal organ functionality (Hu et al., 2016; Drazbo et al., 2018). Importantly, it should be noted that yeast fermentation was able to significantly reduce the content of glucosinolates by about half compared to unfermented rapeseed meal (Table 1), which could be related to the action of microbial enzymes (enzymatic and heat degradation following fermentation; Maribo and Saur, 2012) and suggest that fermentation is a good way to improve the nutritional value of rapeseed meal and prevent interference with nutrient absorption (e.g. minerals). The decrease in glucosinolate contents was also observed by Plaipetch and Yakupitiyage (2013) after fermentation of canola meal with *S. cerevisiae* in a study aimed at evaluating the efficacy of yeast fermentation to reduce the content of glucosinolates and phytic acid.

The enrichment of the chemical composition (protein, fibre, mineral elements, etc.) of canola meal, as well as its effect on performance, feed utilisation, nutrient digestibility and retention was investigated in Nile tilapia. The results of this study confirmed our findings and showed that yeast fermentation increased crude protein and fibre contents of canola meal by 9% and some minerals by 8–32%, while decreased ash and fat content by 1 and 13%, respectively; the latter decrease was explained by the authors by water soaking and decanting (Plaipetch and Yakupitiyage, 2013). Similarly, in the current study, yeast fermentation caused changes in fibre content, increasing it above the level determined in unfermented rapeseed meal.

Literature data have shown that *S. cerevisiae* is rich in fibre (31.4%) (Yamada and Sgarbieri, 2005), which is known for its beneficial effects in the intestine. Fibre reduces nutritive value but improves gastrointestinal peristalsis and promotes the activity of bacteria in the colon, which convert it into lactate, acetate and butyrate, having beneficial effects for the intestinal environment and preventing inflammation (Govers et al., 1999; Fukuda et al., 2002). For example, oral administration of fibre for 6 days significantly reduced inflammation in the colon and increased caecal butyrate levels in rats (Govers et al., 1999; Fukuda et al., 2002). The combination of fibre and *Bifidobacterium infantis* alleviated DSS-induced acute colitis and had an anti-inflammatory effect based on decreasing the synthesis of cytokines and MDA in the colon and increasing short-chain fatty acid concentration in the intestine digesta (Osman et al., 2006). In contrast, the concentration of total polyphenols in our study was lower in fermented compared to unfermented rapeseed meal (54.13 mg GAE/g FRSM vs 88.94 mg GAE/g FRSM), respectively (Table 1).

Diarrhoea, performance and health of piglets

Throughout the experimental period, the incidence of diarrhoea was independent of the treatment, but the number of piglets and days with diarrhoea were higher in the control group than in the group receiving the diet containing fermented rapeseed meal (Table 2). It is noteworthy that the group fed dietary fermented rapeseed did not show any signs of diarrhoea during the first week after weaning and treatment. The trend in diarrhoea incidence was as follows: 4 piglets in the control group (1–3 days) and 0 piglets in the FRSM group during the first week, 6 piglets in the control group (4–7 days) and 2 piglets in the FRSM group (0–3 days) during

Table 2. Ingredients and calculated nutrient content of control and fermented rapeseed meal (FRSM) diets

Ingredients, %	Weaned phase[1]	
	Control diet	FRSM diet
Corn	65.76	64.96
Soybean meal	24.00	15.00
Fermented rapeseed meal	-	10.00
Corn gluten	1.5	1.5
Monocalcium phosphate	0.83	0.48
Limestone	1.40	1.45
NaCl	0.10	0.10
DL-methionine	0.07	0.06
L-lysine	0.23	0.34
Choline premix	0.10	0.10
Mineral vitamin-premix[2]	1.00	1.00
Analysed nutrient content, %		
dry mater	90.62	90.30
crude protein	19.00	18.95
ether extract	1.77	1.70
crude fibre	3.33	3.70
ash	6.48	6.61
Ca	0.87	0.89
P	0.86	0.84
Calculated nutrient content		
digestible protein, %	12.07	11.94
metabolisable energy, kcal/kg	3294	3228
lysine, %	1.20	1.20
digestible lysine, %	1.01	1.01
Met + Cys, %	0.72	0.72
Total	100.00	100.00

[1] body weight 9.06 ± 0.14 kg; [2] mineral-vitamin premix (1%) supplied per kg diet as follows: IU: vit. A 10 000, vit. D_3 2 000, vit. E 30; µg: vit. B_{12} 0.06; mg: vit. K 1.96, vit. B_1 3.84, vit. B_2 14.85, d-pantothenic acid 19.20, niacin 2.94, biotin 24.50, folic acid 0.03, vit. B_6 0.98, vit. C 24.50, Fe 100, Zn 100, Cu 100, Mn 40.30, I 0.38, Se 0.23 and maize starch as carrier

the second week and 5 piglets in the control group (2–5 days) and 1 piglet (2 days) in the FRSM group during the third week. A reduced diarrhoea incidence and significantly fewer days with diarrhoea were also observed by Grela et al. (2019) in piglets from primiparous gilts and multiparous sows receiving 4 to 9% fermented rapeseed feed additive; the latter authors also recorded an increase in litter size and litter weight. Grela et al. (2019) reported no effect on the weight of piglets derived from primiparous gilts at birth and 28 days of age, but they found a significant reduction in piglet weight from multiparous sows. In our study, the fermented rapeseed diet had no significant influence on piglet performance. At the end of the experiment, the weight and average daily weight gain of piglets receiving FRSM had comparable values to piglets receiving the control diet (Table 3). Daily feed intake was also similar

Table 3. Effect of dietary fermented rapeseed meal (FRSM) on pig performance

	Feeding groups			
	Control	FRSM	*P*-value	SEM
Initial weight, kg	9.00	9.00	-	0.158
Final weight, kg	14.50	14.83	0.682	0.618
Daily weight gain, kg	0.262	0.278	0.703	0.021
Daily feed intake, kg	0.738	0.837	-	4.690
Gain:feed, kg:kg	0.355	0.332	-	0.155
Diarrhoea incidence, %	62.50	16.67	0.025	11.82
Total days with diarrhoea, no	16[a]	6[b]	0.001	0.990

SEM – standard error of the mean; [ab] – means within a column with different superscripts are significantly different at $P < 0.05$; ANOVA (one-way) followed by Fisher's tests were performed to analyse the effect of the diets on animal performance (n = 8)

between groups (Table 3). There is less documented research concerning the effect of fermented rape seed meal on pigs and their performance. Few of the existing studies investigated the effect of rapeseed meal fermented mainly using lactic acid bacteria. The most recent studies of Satessa et al. (2020a; b) showed that feeding piglets during three growth periods: 10 days before weaning (18–27 days of age), 28–41 days of age and 28–85 days after weaning with increasing dietary levels (8, 10, 12, 15 or 25%) of rapeseed meal pre-fermented with three lactic acid bacteria resulted in a similar growth performance as in control. Higher performance ($P < 0.01$) than in control was observed for the period of 28–41 days of age with 8% FRM inclusion. The use of lactic bacteria-fermented rapeseed meal in the diet of other species (broiler chickens, duck, fish) resulted in better performance and health (Chiang, 2010; Fazhi et al., 2011; Plaipetch and Yakupitiyage, 2011; 2013). In contrast to these positive results, Maribo and Saur (2012) reported lower gains, poor feed conversion ratio and no effect on health in pigs after weaning (9–30 kg) fed 15% rape seed cakes fermented with lactic acid bacteria, bran, soy molasses and potato peel.

Much more research has been carried out in the past and currently on the possibility of replacing soybean meal with unfermented rapeseed meal and the effects on pig performance, protein digestibility, etc. Contrary to previous literature results, which suggested that replacing soybean meal with canola meal (rapeseed hybrid) affected pig performance (Bell, 1993; Mejicanos et al., 2016), recent studies of Landero et al. (2011; 2012; 2018), and Pérez de Nanclares et al. (2019) did not find significant differences in the growth and feed performance of weaned piglets when soybean meal was substituted

with 5, 10, 15, 20 and 30% solvent-extracted canola meal during 28 days of the trial; however, a decrease in the apparent total tract digestibility (ATTD) of dry matter (DM), energy and crude protein was observed when the inclusion percentage was increased Do et al. (2017) and Mejicanos et al. (2016) also demonstrated that the use of different dietary levels of rapeseed meal or canola meal (up to 8%) exerted no detrimental effects on weaned pigs' performance and that both could partially or completely replace soybean meal.

The analysis of plasma biochemistry parameters revealed no significant differences between control and fermented rapeseed diet in plasma biochemistry (Table 4), which was consistent with findings reported by Satessa et al. (2020a), who fed weaned piglets with pre-fermented rapeseed diet using lactic acid bacteria. On the other hand, the latter results were contrary to the outcomes of Czech et al. (2021), who observed an increase in these parameters (ALP, AST, ALT and LDH) in comparison to control and other experimental groups, which according to the authors, could be due to an increase in amino acid metabolism.

Table 4. Effect of dietary fermented rapeseed meal (FRSM) on biochemical parameters of piglet blood plasma

	Feeding groups			
	Control	FRSM	P-value	SEM
ALAT, U/l	43.77	39.66	0.289	1.869
ASAT, U/l	50.00	49.15	0.813	1.693
ALP, U/l	148.58	130.06	0.141	6.206
GGT, U/l	26.01	23.18	0.467	1.852
Total protein, g/l	57.92	54.67	0.135	0.158
Total bilirubin, µmol/l	2.74	2.91	0.727	0.006
Albumin, g/l	30.0	26.7	0.186	0.128
Urea, mmol/l	3.14	3.16	0.895	0.611
Creatinine, µmol/l	108.29	108.59	0.964	0.05
Cholesterol, mmol/l	2.03	1.97	0.951	2.094

ALAT – alanine transaminase, ASAT – aspartate transaminase, ALP – alkaline phosphatase, GGT – gamma-glutamyltransferase; SEM – standard error of the mean; ($P > 0.05$); ANOVA (one-way) followed by Fisher's tests were performed to analyse the effect of the diets on animal performance (n = 8)

Inflammation

During weaning, piglets are exposed to environmental and dietary changes that modify intestinal morphology and function (decrease in transepithelial electrical resistance and increase in barrier permeability) (de Groot et al., 2021). These disturbances, along with infiltration of T lymphocytes into the colon and sometimes small intestine, may activate the intestinal inflammatory response by upregulating the expression of pro-inflammatory cytokine genes (Pié et al., 2004; Satessa et al., 2020a; Long et al.,

2021). In our study, interleukins IL-1ß, IL-6, IL-8, TNF-α and IFN-γ – early nonspecific mediators of inflammation (Pié et al., 2004) were further determined to assess intestinal inflammation. Our results showed a decrease in the most potent pro-inflammatory cytokines, i.e. TNF-α ($P < 0.03$) and IL-1ß ($P < 0.05$), in colon samples from pigs fed the diet with fermented rapeseed meal (Figure 1). TNF-α concentration also decreased in the jejunum

in piglets after weaning (Satessa et al., 2020b). Yeast were shown to inhibit the activation of NF-κB and NF-κB-mediated IL-8 gene expression in intestinal epithelial cells, resulting in decreased inflammation (Sougioultzis et al., 2006). In the present study, reduced IL-8 and IL-6 levels were also found in the ileum ($P < 0.0009$ and $P < 0.03$) of piglets fed FRSM, while no differences were recorded in the jejunum. Interestingly, colon IL-6 concentration increased
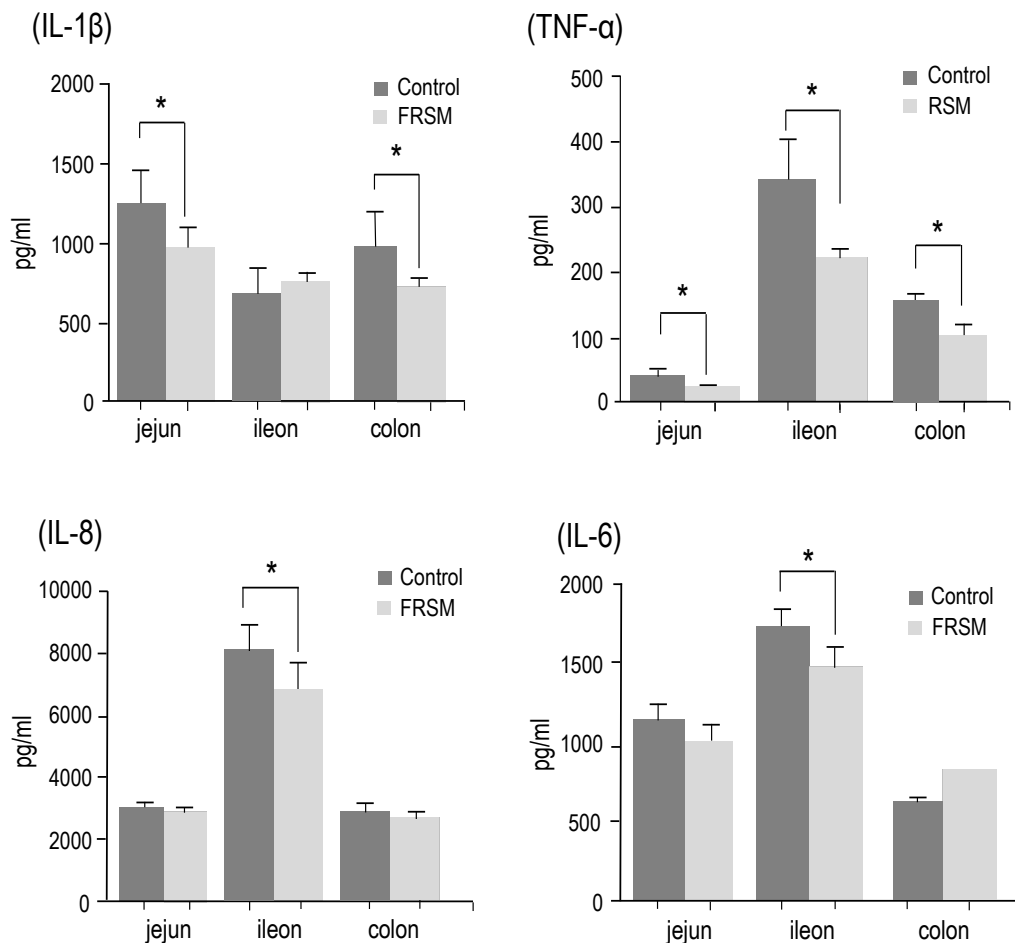

Figure 1. Effect of fermented rapeseed meal (FRSM) diet on intestinal inflammatory markers. The means value ± SEM were calculated and presented as a histogram (n = 8). Statistical analysis was performed using one-way ANOVA followed by Fisher's test (*$P < 0.05$). TNF-α – tumour necrosis alpha, IL-1β – interleukine 1 beta, IL-6 – interleukine 6, IL-8 – interleukine 8

($P < 0.04$), while IL-1ß only showed a reduced tendency ($P < 0.1$) in the jejunum in the FRSM diet group, indicating the modulatory capacity of fermented rapeseed meal in reducing weaning-associated intestinal inflammation. Therefore, it was concluded that the anti-inflammatory effect was due to yeast controlling and limiting T cell infiltration in the small intestine, and especially in the colon (Satessa et al., 2020b). The same authors found immune cell infiltration only in the stroma of intestinal segments in another study aimed at investigating the potential of fermented rapeseed meal as a replacer of medicinal zinc oxide

($P = 0.0170$) in response to the 10% FRSM diet. Satessa et al. (2020b) also reported elevated serum IL-6 levels in pigs fed a diet supplemented with 8% or 10% FRM compared to control; however, in contrast to our study, the latter authors also recorded an increase in the levels of serum immunoglobulins (IgG, IgM, IgA) 14 days after weaning.

The effectiveness of using nutrition as a strategy to control inflammation is becoming increasingly important. In this context, the application of plant extracts with anti-inflammatory properties as alternatives to antibiotics was described by Zhou et al.

(2014), Long et al. (2019) and Mendivil et al. (2019), who demonstrated, among others, that capsaicin, a bioactive compound from chili pepper, reduced the levels of pro-inflammatory cytokines TNF-α, IL-1ß and IL-6 and increased immune response in pigs fed a diet containing natural capsicum extract (80 mg/kg). Reduction in inflammation was modulated by downregulation of the NF-κB pathway (Zhou et al., 2014).

Dietary interventions to modulate inflammation and oxidative status in pigs have also proposed probiotics/immunobiotics as alternative sources to avoid high levels of inflammation above the protective limit (Suda et al., 2021). Among immunobiotics, lactobacillus strains demonstrated the ability to differentially regulate inflammatory and anti-inflammatory cytokines and to confer protection against infection (Suda et al., 2021). Various studies found that oral administration of *Lacticaseibacillus casei* CRL431, *Lactiplantibacillus plantarum* CRL1506, and *Lacticaseibacillus rhamnosus* CRL1505 decreased IL-8 and MCP-1 levels and increased IFN-γ and IL-10 levels in the gut of mice with intestinal and viral infections (Nakanishi et al., 2006; Salva et al., 2010; Villena et al., 2018). Similarly, *Lactobacillus delbrueckii* subsp. *delbrueckii* TUA4408L modulated the inflammatory cytokine profile in ETEC-challenged intestinal epithelial cells and weaned piglets by reducing the expression and levels of IL-6, IL-8 and MCP-1 cytokines (Suda et al., 2021).

Antioxidant status and humoral immune response

We further investigated the antioxidant status and immune response as assessed by antioxidant enzyme activities and immunoglobulin levels in serum and intestine segments, knowing that weaning stress could affect these two important piglet health parameters (Campbell et al., 2013; Long et al., 2021). Rapeseed meal is a rich source of several active components with antioxidant properties, such as vitamin E, vitamin B complex, minerals, unsaturated fatty acids and phenolic acids – stimulators of antioxidant enzyme

activity (Nićiforović and Abramovič, 2014; Chen et al., 2018). Moreover, several metabolic by-products are formed during yeast fermentation, including enzymes, vitamins and other nutrients and important co-factors (Yang et al., 2016). In addition, yeast wall contains polysaccharides that have antioxidant properties due to their polymeric structure trapping free radicals like β-glucans (Pourahmad et al., 2011). Yang et al., (2016) showed that feeding weaned piglets with yeast products containing β-glucans enhanced serum and intestinal antioxidant indices (total antioxidant capacity, SOD and CAT). In our experiment (Table 5), the diet containing 10% FRSM significantly increased SOD ($P < 0.0001$) and CAT ($P < 0.02$) activities at the systemic level in plasma, but not in the intestine (Table 6). Conversely, piglets receiving either a diet containing 8% fermented rapeseed meal or a combination of fermented rapeseed and fermented soybean meal showed no increase in blood plasma SOD and CAT levels, but had significantly enhanced local activity of these enzymes in the jejunum (Czech et al., 2021). In turkeys, the inclusion of 15% fermented rapeseed cakes in the diet did not modify SOD and CAT and decreased

Table 5. Effect of dietary fermented rapeseed meal (FRSM) on antioxidant indices and humoral immune response in pig plasma

| | Feeding groups | | | |
	Control	FRSM	*P*-value	SEM
Plasma antioxidant indices				
total antioxidant capacity, U/ml	0.706	0.708	0.750	0.00
superoxide dismutase, U/ml	4.623[a]	6.188[b]	0.0001	0.26
catalase, U/ml	11.142[a]	18.228[b]	0.0001	3.16
glutathione peroxidase, U/ml	1.933	1.670	0.253	0.10
TBARS/MDA, nmol/ml	3.913[a]	2.938[b]	0.022	0.18
Plasma humoral response, mg/ml				
IgA	0.647	0.648	0.511	0.08
IgM	1.603	1.714	0.501	0.11
IgG	6.332	5.501	0.181	0.53

TBARS/MDA – thiobarbituric acid substances/malondialdehyde, Ig – immunoglobulin; SEM – standard error of the mean; [ab] – means within a column with different superscripts are significantly different at $P < 0.05$. ANOVA (one-way) followed by Fisher's tests were performed to analyse the effect of the diets on animal performance (n = 8)

Table 6. Effect of dietary FRSM on antioxidant enzyme activity in pig intestinal tissue

| | SOD, U/g tissue | | | | CAT, µmol/min/g tissue | | | | GPx, µmol/min/g tissue | | | |
	Control	FRSM	*P*-value	SEM	Control	FRSM	*P*-value	SEM	Control	FRSM	*P*-value	SEM
Jejunum	374.21	368.68	0.73	7.58	2.55	3.36	0.25	0.36	3.96	3.25	0.32	0.41
Ileum	356.56	347.94	0.58	7.38	2.25	2.66	0.38	0.22	3.30	3.61	0.64	0.32
Colon	334.06	347.84	0.18	2.60	4.09	3.98	0.76	0.18	2.09	1.84	0.45	0.16

SOD – superoxide dismutase, CAT – catalase, GPx – glutathyon peroxidase; SEM – standard error of the mean; ($P > 0.05$); ANOVA (one-way) followed by Fisher's tests were performed to analyse the effect of the diets on animal performance (n = 8)

GPx activities in blood, which was a worse result compared to unfermented rapeseed (Drażbo et al., 2018).

Our 10% FRSM diet did not differentially modify plasma immunoglobulin concentrations in piglets from the FRSM group compared to control (Table 5). The cytokine IL-6, which together with IL-4, stimulate antibody production (Mizumachi et al., 2009) was also not modified in plasma and showed a lower intestinal level under the influence of the diet with fermented rapeseed meal, which probably explains the lack of alterations in immunoglobulin synthesis. The lack of effect on non-specific humoral immune response was also reported by Long et al. (2019) in piglets fed a diet supplemented with *Forsythia suspense* extract used as a potential non-antibiotic supplement. In contrast, these authors showed that dietary supplementation with *Forsythia suspense* extract enhanced total antioxidant capacity, superoxide dismutase and catalase levels in serum of piglets after 28 days of treatment compared to control. Satessa et al. (2020b) also investigated the effect of different dietary levels of fermented rapeseed meal in piglets after weaning and detected increased serum concentrations of immunoglobulins (IgG, IgM, IgA) and IL-6 in pigs fed diets supplemented with 8 and 10% lactobacillus-pre-fermented rapeseed meal. This was probably the piglet's response to lactic acid bacteria, which are immune stimulators and active components of fermented rapeseed meal (Satessa et al., 2020b). A similar response to fermented feed, i.e. higher serum levels of immunoglobulins was obtained by Zhu et al. (2017), who fed piglets after weaning with 5, 10 and 15% lactobacilli-fermented soybean meal. Additionally, a fermented liquid diet containing probiotics (*Lactobacillus plantarum* LQ80) stimulated systemic antibody (IgM and IgG) production in piglets after weaning without causing inflammatory reactions (Mizumachi et al., 2009). Our results regarding the antioxidant status at the intestinal tissue level showed that SOD activity was not significantly modulated by the administration of fermented rapeseed meal. In contrast, the results showed a trend towards an increase in catalase activity in the jejunum ($P = 0.08$) of piglets fed the FRSM diet (Table 6). No effect of FRSM addition on GPx activity in the ileum and colon was observed (Table 6).

Oxidative stress accompanying inflammation during weaning period is associated with the production of free radicals that cause cellular damage (Valko et al., 2005). Unsaturated cellular lipid membrane is one of the main peroxidation targets of free radicals (Yoshida et al., 2015). There are studies showing that dietary inclusion of fermented ingredients can diminish the effects of oxidative stress. Indeed, in our experiment, after the administration of the FRSM diet, the level of thiobarbituric acid reactive substances/malodialdehyde (TBARS/MDA), markers of lipid peroxidation induced by oxidative stress, was significantly decreased in blood plasma ($P = 0.022$) and two intestinal segments, jejunum ($P = 0.028$) and colon ($P = 0.003$), suggesting that the diet with FRSM has the capacity to diminish lipid peroxidation during weaning crisis, which may be due to the anti-oxidant and scavenging properties of the active components present in rapeseed meal and yeast (Figure 2A). Similarly to our results, an improvement in the redox status in plasma (increased antioxidant potential, and albumins contents and CAT activity), liver and jejunum (increased SOD and CAT activity), as well as decreased MDA in plasma, liver and jejunum was found by Czech et al. (2021) in weaned piglets fed a diet containing 6% fermented rapeseed meal and 2% fermented soybean meal, respectively. The authors argued that these improved results were due to the fermentation process, increasing the content of low-molecular weight peptides with antioxidant properties. Indeed, Xue et al. (2009a; b) reported that peptides from rapeseed protein hydrolysate showed dose-dependent anti-oxidative properties and inhibited MDA and superoxide anion generation. In another study (Xue et al., 2009b), the same authors showed that protein hydrolysate (RSCH) obtained from rapeseed (cultivar Huaza3) was able to improve immune function (phagocytic capacity of celiac macrophages and delayed-type hypersensitivity) to increase serum SOD activity by reducing the formation of free radicals and thiobarbituric acid reactive substances in serum, and recommend the use of rapeseed in human nutrition as a source of bioactive peptides with antitumor an anti-oxidative properties.

Diamine oxidase is a biomarker commonly used for monitoring intestinal barrier integrity that plays a key role in the body's defence against pathogen invasion into the mucosa (Long et al., 2021; Zou et al., 2021). It is produced exclusively in the small intestine. The damage to the intestinal barrier led to the release and increase of DAO plasma levels and decreased intestinal concentration (Xiao et al., 2014). In the current study, pigs fed fermented rapeseed diet showed a significant decrease in plasma DAO concentration, suggesting that the intestinal barrier was not affected by the FRSM diet (Figure 2B).
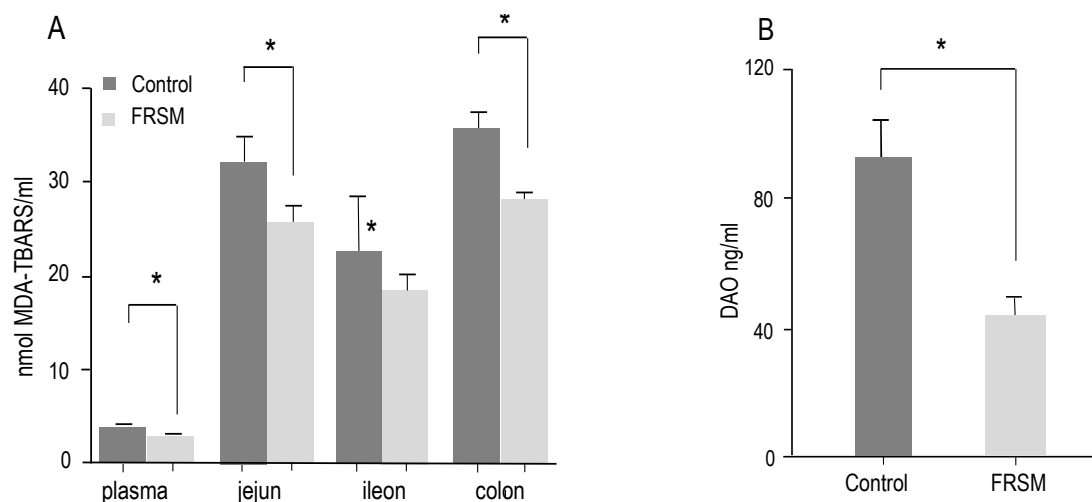
Figure 2. Effect of fermented rapeseed meal (FRSM) diet on oxidative stress and intestinal barrier integrity. The mean value ± SEM were calculated and presented as a histogram (n = 8). Statistical analysis was performed using one-way ANOVA followed by Fisher's test (*$P < 0.05$); A) TBARS/MDA – thiobarbituric acid substances/malondialdehyde; B) DAO – diamine oxidase

Signalling pathway

Inflammation and oxidative stress are mainly regulated by the central mediator NF-κB and Nrf2 signalling pathways. NF-κB promotes inflammation by regulating the transcription of genes coding for cytokines and chemokines, immunoreceptors, cell-adhesion molecules, growth factors, etc. (Gilmore, 2006), while Nrf2 plays an essential role in the regulation of the antioxidant responsive element (ARE) and induction of genes encoding phase II detoxifying and anti-oxidative stress enzymes (La Marca et al., 2013). Many studies have demon

colonic tissue of mice lacking Nrf2 (-/-). In rats, a diet supplemented with multiple antioxidants reduced the increase in oxidative stress, while inhibiting NF-κB (Batumalaie et al., 2013). Our results (Table 7) showed that the level of the active form of NF-κB (p65) decreased in the ileum ($P = 0.03$) under the influence of a diet containing fermented rapeseed meal, while an increase in Nrf2 expression was observed in the ileal tissue, ($P = 0.007$) which explained the decrease in the concentration of pro-inflammatory cytokines and a slight increase in the activity of antioxidant enzymes.

Table 7. Effect of of dietary fermented rapeseed meal (FRSM) addition on signalling pathway indices in pig intestinal segments

	NF-κb, U.A.				Nrf2, U.A.			
	Control	FRSM	*P*-value	SEM	Control	FRSM	*P*-value	SEM
Jejunum	0.402	0.345	0.16	0.01	0.191	0.208	0.21	0.02
Ileum	0.498[a]	0.404[b]	0.03	0.03	0.242[a]	0.277[b]	0.007	0.01
Colon	0.190[a]	0.162[b]	0.17	0.02	0.367	0.475	0.15	0.01

NF-κB – nuclear factor kappa B, Nrf2 – nuclear factor erythroid 2-related factor 2; SEM – standard error of the mean; [ab] – means within a column with different superscripts are significantly different at $P < 0.05$; ANOVA (one-way) followed by Fisher's tests were performed to analyse the effect of the diets on animal performance (n = 8)

strated the existence of a cross-talk between NF-κB and Nrf2 signalling pathways in response to inflammation and oxidative stress (Saw et al., 2010), indicating that Nrf2 activation suppresses inflammation by inhibiting NF-κB induction by regulating redox balance (Lin et al., 2008; Wang et al., 2010a; b). In an elegant study, Khor et al. (2006) showed that the levels of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) and mediators (cyclooxygenase 2, iducible nitric oxide synthase 2) were significantly increased by sodium dextran sulphate (1%), while antioxidant/phase II detoxifying enzymes were decreased in

Conclusions

In conclusion, the present study showed that fermentation of rapeseed meal with *S. cerevisiae* enriched its composition with crude protein, crude fibre and some minerals, and was an effective way to reduce anti-nutritional glucosinolate levels. The results demonstrated the potential effectiveness of rapeseed fermented diet in improving the intestinal health status of piglets by modulating significant processes during the weaning period, such as the pro-inflammatory oxidative response and intes-

tinal barrier integrity. Further studies are needed to investigate different dosages for incorporating fermented rapeseed meal into the pig diet.

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Conflict of interest

The Authors declare that there is no conflict of interest.

References

Batumalaie K., Safi S.Z., Yusof K.M., Ismail I.S., Sekaran S.D., Qvist R., 2013. Effect of gelam honey on the oxidative stress-induced signaling pathways in pancreatic hamster cells. Int. J. Endocrinol. 2013, 367312, 1–11, https://doi.org/10.1155/2013/367312

Bell J.M., 1993. Factors affecting the nutritional value of canola meal: a review. Can. J. Anim. Sci. 73, 689–697, https://doi.org/10.4141/cjas93-075

Campbell J.M., Crenshaw J.D., Polo J., 2013. The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol. 4, 1–4, https://doi.org/10.1186/2049-1891-4-19

Chen C., de Nanclares M.P., Kurtz J.F., et al., 2018. Identification of redox imbalance as a prominent metabolic response elicited by rapeseed feeding in swine metabolome. J. Anim. Sci. 96, 1757–1768, https://doi.org/10.1093/jas/sky080

Chiang G., Lu W.Q., Piao X.S., Piao, Hu J.K., Gong L.M., Thacker P.A., 2010. Effects of feeding solid-state fermented rapeseed meal on performance, nutrient digestibility, intestinal ecology and intestinal morphology of broiler chickens. Asian-Australas. J. Anim. Sci. 23, 263–271, https://doi.org/10.5713/ajas.2010.90145

Chuang W.Y., Lin L.J., Hsieh Y.C., Chang S.C., Lee T.T., 2021. Effects of *Saccharomyces cerevisiae* and phytase co-fermentation of wheat bran on growth, antioxidation, immunity and intestinal morphology in broilers. Anim. Biosci. 34, 1157–1168, https://doi.org/10.5713/ajas.20.0399

Czech A., Sembratowicz I., Kiesz M., 2021. The effects of a fermented rapeseed or/and soybean meal additive on antioxidant parameters in the blood and tissues of piglets. Animals 11, 1646, 1–10, https://doi.org/10.3390/ani11061646

de Groot N., Fariñas F., Cabrera-Gómez C.G., Pallares F.J., Ramis G., 2021. Weaning causes a prolonged but transient change in immune gene expression in the intestine of piglets. J. Anim. Sci. 99, 1–12, https://doi.org/10.1093/jas/skab065

Do S.H. Kim B.O., Fang L.H., You D.H., su Hong J., Kim Y.K., 2017. Various levels of rapeseed meal in weaning pig diets from weaning to finishing periods. Asian-Australas. J. Anim. Sci. 30, 1292–1302, https://doi.org/10.5713/ajas.16.0953

Dragomir C., Rinne M., Ruiz D.R.Y., 2015. Novel coproducts from corn milling and their use in ruminants' nutrition. Turk. J. Vet. Anim. 39, 245–253, https://doi.org/10.3906/vet-1412-27

Drażbo A.A., Juśkiewicz J., Józefiak A., Konieczka P., 2020. The fermentation process improves the nutritional value of rapeseed cake for turkeys-effects on performance, gut bacterial population and its fermentative activity. Animals 10, 1711, https://doi.org/10.3390/ani10091711

Drazbo A, Ognik K , Zaworska A, Ferenc K, Jankowski J., 2018. The effect of raw and fermented rapeseed cake on the metabolic parameters, immune status, and intestinal morphology of turkeys. Poult Sci. 97, 3910–3920, https://doi.org/10.3382/ps/pey250

EC (European Commission), 2006. https://ec.europa.eu/commission/presscorner/detail/en/IP_05_1687

Fazhi X., Lvmu L., Jiaping X., Kun Q., Zhide Z., Zhangyi L., 2011. Effects of fermented rapeseed meal on growth performance and serum parameters in ducks. Asian-Australas. J. Anim. Sci. 24, 678–684, https://doi.org/10.5713/ajas.2011.10458

Feng H., Qu H., Liu Y., Shi Y., Wu S., Bao W., 2020. Effect of fermented soybean meal supplementation on some growth performance, blood chemical parameters, and fecal microflora of finishing pigs. R. Bras. Zootec. 49, https://doi.org/10.37496/rbz4920190096

Fukuda M. Kanauchi O., Araki Y., et al., 2002. Prebiotic treatment of experimental colitis with germinated barley foodstuff: a comparison with probiotic or antibiotic treatment. Int. J. Mol. Med. 9, 65–70, https://doi.org/10.3892/ijmm.9.1.65

Gilmore T.D., 2006. Introduction to NF-κB: players, pathways, perspectives. Oncogene 25, 6680-6684, https://doi.org/10.1038/sj.onc.1209954

Govers M.J., Gannon N.J., Dunshea F.R., Gibson P.R., Muir J.G., 1999. Wheat bran affects the site of fermentation of resistant starch and luminal indexes related to colon cancer risk: a study in pigs. Gut 45, 840–847, https://doi.org/10.1136/gut.45.6.840

Grela E.R., Czech A., Kiesz M., Wlazło Ł., Nowakowicz-Dębek B., 2019. A fermented rapeseed meal additive: effects on production performance, nutrient digestibility, colostrum immunoglobulin content and microbial flora in sows. Anim. Nutr. 5, 373–379, https://doi.org/10.1016/j.aninu.2019.05.004

Hong K.J., Lee C.-H., Kim S.W., 2004. *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. J. Med. Food 7, 430–435, https://doi.org/10.1089/jmf.2004.7.430

Hu Y., Wang Y., Li A., Wang Z., Zhang X., Yun T., Qiu L., Yin Y., 2016. Effects of fermented rapeseed meal on antioxidant functions, serum biochemical parameters and intestinal morphology in broilers. Food Agric. Immunol. 27, 182–193, https://doi.org/10.1080/09540105.2015.1079592

ISO (International Organization for Standardization), 2010. Standardized Bulletin, SR ISO 6496/2001, http://www.asro.ro

Khor T.O., Huang M.-T., Kwon K.H., Chan J.Y., Reddy B.S., Kong A.-N., 2006. Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis. Cancer Res. 66, 11580–11584, https://doi.org/10.1158/0008-5472.CAN-06-3562

La Marca M., Beffy P., Pugliese A., Longo V., 2013. Fermented wheat powder induces the antioxidant and detoxifying system in primary rat hepatocytes. PLOS ONE 8, e83538, https://doi.org/10.1371/journal.pone.0083538

Lallès J.-P., Boudry G., Favier C., Le Floc'h N., Luron I., Montagne L., Oswald I.P., Pie C., Seve B., 2004. Gut function and dysfunction in young pigs: physiology. Anim. Res. 53, 301–316, https://doi.org/10.1051/animres:2004018

Landero J.L., Beltranena E., Cervantes M., Araiza A.B., Zijlstra R.T., 2012. The effect of feeding expeller-pressed canola meal on growth performance and diet nutrient digestibility in weaned pigs. Animal Feed Sci. Technol. 171, 240–245, https://doi.org/10.1016/j.anifeedsci.2011.11.004

Landero J.L., Beltranena E., Cervantes M., Morales A., Zijlstra R.T., 2011. The effect of feeding solvent-extracted canola meal on growth performance and diet nutrient digestibility in weaned pigs. Anim. Feed Sci. Technol. 170, 136–140, https://doi.org/10.1016/j.anifeedsci.2011.08.003

Landero J.L., Wang L.F., Beltranena E., Bench C.J., Zijlstra R.T., 2018. Feed preference of weaned pigs fed diets containing soybean meal, *Brassica napus* canola meal, or *Brassica juncea* canola meal. J. Anim. Sci. 96, 600–611, https://doi.org/10.1093/jas/skx052

Lee J.O., Park M.H., Choi Y.H., Ha Y.L., Ryu C.H., 2007. New fermentation technique for complete digestion of soybean protein. J. Microbiol. Biotechnol. 17, 1904–1907

Li P., Lyu Z., Wang L., Huang B., Lai C., 2020. Nutritive values of double-low rapeseed expellers and rapeseed meal with or without supplementation of multi-enzyme in pigs. Can. J. Anim. Sci. 100, 729–738, https://doi.org/10.1139/cjas-2019-0097

Lin W., Wu R.T., Wu T., Khor T.-O., Wang H., Kong A.-N., 2008. Sulforaphane suppressed LPS-induced inflammation in mouse peritoneal macrophages through Nrf2 dependent pathway. Biochemical pharmacology 76, 967–973, https://doi.org/10.1016/j.bcp.2008.07.036

Long S., Liu L., Liu S., Mahfuz S., Piao X., 2019. Effects of Forsythia Suspense extract as an antibiotics substitute on growth performance, nutrient digestibility, serum antioxidant capacity, fecal Escherichia coli concentration and intestinal morphology of weaned piglets. Animals 9, 729, 1–12, https://doi.org/10.3390/ani9100729

Long S., Ma J., Piao X., Li Y., Rasmussen S.H., Liu L., 2021. Enzyme-treated soybean meal enhanced performance via improving immune response, intestinal morphology and barrier function of nursery pigs in antibiotic free diets. Animals 11, 2600, 1–12, https://doi.org/10.3390/ani11092600

Maribo H., Saur C., 2012. Fermented rapeseed for weaners. Pig Research Centre. Trial report no. 942

Marin D., Bulgaru C.V., Anghel C.A., Pistol G.C., Dore M.I., Palade M.L., Taranu I., 2020. Grape seed waste counteracts aflatoxin B1 toxicity in piglet mesenteric lymph nodes. Toxins 12, 800, 1–14, https://doi.org/10.3390/toxins12120800

Marin D.E., Pistol G.C., Neagoe I.V., Calin L., Taranu I., 2013. Effects of zearalenone on oxidative stress and inflammation in weanling piglets. Food Chem. Toxicol. 58, 408–415, 1–14, https://doi.org/10.1016/j.fct.2013.05.033

Mejicanos G., Sanjayan N., Kim I.H., Nyachoti C.M., 2016. Recent advances in canola meal utilization in swine nutrition. J. Anim. Sci. Technol. 58, 7, 1–13, https://doi.org/10.1186/s40781-016-0085-5

Mendivil E.J., Sandoval-Rodriguez A., Meza-Rios A., Zuniga-Ramos L., Dominguez-Rosales A, Mercado M.V., Sanchez-Orozco L., Santos-Garcia A., Armendariz-Boruda J., 2019. Capsaicin induces a protective effect on gastric mucosa along with decreased expression of inflammatory molecules in a gastritis model. J. Funct. Foods 59, 345–351, https://doi.org/10.1016/j.jff.2019.06.002

Mizumachi K., Aoki R., Ohmori H., Saeki M., Kawashima T., 2009. Effect of fermented liquid diet prepared with *Lactobacillus plantarum* LQ80 on the immune response in weaning pigs. Animal 3, 670–676, https://doi.org/10.1017/S1751731109003978

Nakanishi Y., Murashima K., Ohara H. et al., 2006. Increase in terminal restriction fragments of *Bacteroidetes*-derived 16S rRNA genes after administration of short-chain fructooligosaccharides. Appl. Environ. Microbiol. 72, 6271–6276, https://doi.org/10.1128/AEM.00477-06

Nićiforović N., Abramovič H., 2014. Sinapic acid and its derivatives: natural sources and bioactivity. Compr. Rev. Food Sci. Foofd Saf. 13, 34–51, https://doi.org/10.1111/1541-4337.12041

NRC (National Research Council), 2012. Nutrient requirements of swine. 11th Revised Edition. National Academies Press, Washington, DC (USA), https://doi.org/10.17226/13298

Onarman Umu Ö.C., Fauske A.K., Akesson C.P, Perez de Nanclares M., Sorby R., McLean Press C., Overland M., Sorum H., 2018. Gut microbiota profiling in Norwegian weaner pigs reveals potentially beneficial effects of a high-fiber rapeseed diet. PLOS ONE 13, e0209439, https://doi.org/10.1371/journal.pone.0209439 Osman N., Adawi D., Molin G., Berggren A., Jeppsson B., 2006. *Bifidobacterium infantis* strains with and without a combination of oligofructose and inulin (OFI) attenuate inflammation in DSS-induced colitis in rats. BMC Gastroenterol. 6, 31, https://doi.org/10.1186/1471-230X-6-31

Palade L.M., Habeanu M., Marin D.E., Chedea V.S., Pistol G.C., Grosu I.A., Gheorghe A., Ropota M., Taranu I., 2019. Effect of dietary hemp seed on oxidative status in sows during late gestation and lactation and their offspring. Animals 9, 194, https://doi.org/10.3390/ani9040194

Pérez de Nanclares M., Marcussen C., Tauson A.-H., Hansen J.Q., Kjos N.P., Mydland L.T., Bach Knaussen K.E., Overland M., 2019. Increasing levels of rapeseed expeller meal in diets for pigs: effects on protein and energy metabolism. Animal 13, 273–282, https://doi.org/10.1017/S1751731118000988

Pié S., Lalles J.P., Blazy F., Laffitte J., Seve B., Oswald I.P., 2004. Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. J. Nutr. 134, 641–647, https://doi.org/10.1093/jn/134.3.641

Pistol G.C., Marin D.E., Rotar M.C., Ropota M., Taranu I., 2020. Bioactive compounds from dietary whole grape seed meal improved colonic inflammation via inhibition of MAPKs and NF-*k*B signaling in pigs with DSS induced colitis. J. Funct. Foods 66, 103708, https://doi.org/10.1016/j.jff.2019.103708

Plaipetch P., Yakupitiyage A., 2011. Use of yeast-fermented canola meal to replace fishmeal in the diet of Asian sea bass *Lates Calcarifer* (Bloch, 1790). J. Aquac. Res. Development 03, https://doi.org/10.4172/2155-9546.1000125

Plaipetch P., Yakupitiyage A., 2013. Effect of replacing soybean meal with yeast-fermented canola meal on growth and nutrient retention of Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758). Aquac. Res. 45, 1744–1753, https://doi.org/10.1111/are.12119

Pourahmad J., Shaki F., Tanbakosazan F., Ghalandari R., Ettehadi H.A., Dahaghin E., 2011. Protective effects of fungal β-(1→3)-D-glucan against oxidative stress cytotoxicity induced by depleted uranium in isolated rat hepatocytes. Hum. Exp. Toxicol. 30, 173–181, https://doi.org/10.1177/0960327110372643

Salva S., Villena J., Alvarez S., 2010. Immunomodulatory activity of *Lactobacillus rhamnosus* strains isolated from goat milk: impact on intestinal and respiratory infections. Int. J. Food Microbiol. 141, 82–89, https://doi.org/10.1016/j.ijfoodmicro.2010.03.013

Satessa G.D., Tamez-Hidalgo P., Hui Y., Cieplak T., Krych L., Kjaerulff S., Brunsgaard G., Nielsen D.S., Nielsen M.O., 2020a. Impact of dietary supplementation of *lactic acid bacteria* fermented rapeseed with or without macroalgae on performance and health of piglets following omission of medicinal zinc from weaner diets. Animals 10, 137, 1–20, https://doi.org/10.3390/ani10010137

Satessa G.D., Tamez-Hidalgo P., Kjaerulff S., Vargas-Bello-Perez E., Dhakal R., Nielsen M.O., 2020b. Effects of increasing doses of lactobacillus pre-fermented rapeseed product with or without inclusion of macroalgae product on weaner piglet performance and intestinal development. Animals 10, 559, 1–17, https://doi.org/10.3390/ani10040559

Saw C.L., Wu Q., Kong A.N., 2010. Anti-cancer and potential chemopreventive actions of ginseng by activating Nrf2 (NFE2L2) anti-oxidative stress/anti-inflammatory pathways. Chin. Med. 5, 37, 1–7, https://doi.org/10.1186/1749-8546-5-37

Sougioultzis S., Simeonidis S., Bhaskar K.R., Chen X., Anton P.M., Keates S., Pothoulakis C., Kelly C.P., 2006. *Saccharomyces boulardii* produces a soluble anti-inflammatory factor that inhibits NF-κB-mediated IL-8 gene expression. Biochem. Biophys. Res.. Commun. 343, 69–76, https://doi.org/10.1016/j.bbrc.2006.02.080

Suda Y., Sasaki N., Kagawa K. et al., 2021. Immunobiotic feed developed with *Lactobacillus delbrueckii* subsp. *delbrueckii* TUA4408L and the soymilk by-product Okara improves health and growth performance in pigs. Microorganisms 9, 921, 1–19, https://doi.org/10.3390/microorganisms9050921

Taranu I., Habeanu M., Gras M.A., Pistol G.C., Lefter N., Palade M., Ropota M., Chdea V.S., Marin D.E., 2018. Assessment of the effect of grape seed cake inclusion in the diet of healthy fattening-finishing pigs. J. Anim. Physiol. Anim. Nutr. 102, e30–e42, https://doi.org/10.1111/jpn.12697

Taranu I., Marin D.E., Pistol G.C., Motiu M., Pelinescu D., 2015. Induction of pro-inflammatory gene expression by *Escherichia coli* and mycotoxin zearalenone contamination and protection by a Lactobacillus mixture in porcine IPEC-1 cells. Toxicon 97, 53–63, https://doi.org/10.1016/j.toxicon.2015.01.016

Untea A.E., Criste R.D., Vladescu L., 2012. Development and validation of a microwave digestion - FAAS procedure for Cu, Mn and Zn determination in liver. Rev. Chim. 63, 341–346

Valko M., Morris H., Cronin T.D., 2005. Metals, toxicity and oxidative stress. Curr. Med. Chem. 12, 1161–1208, https://doi.org/10.2174/0929867053764635

Villena J., Aso H., Rutten V.P.M.G., Takahashi H., van Eden W., Kitazawa H., 2018. Immunobiotics for the bovine host: their interaction with intestinal epithelial cells and their effect on antiviral immunity. Front. Immunol. 9, 326, 3–10, https://doi.org/10.3389/fimmu.2018.00326

Wang H., Khor T.O., Saw C.L.L., Lin W., Wu T., Huang Y. Kong A.T., 2010a. Role of Nrf2 in suppressing LPS-induced inflammation in mouse peritoneal macrophages by polyunsaturated fatty acids docosahexaenoic acid and eicosapentaenoic acid. Mol. Pharm. 7, 2185–2193, https://doi.org/10.1021/mp100199m

Wang R., Shaarani S.M., Godoy L.C., Melikoglu M., Vergara C.S., Koutinas A., Webb C., 2010b. Bioconversion of rapeseed meal for the production of a generic microbial feedstock. Enzyme Microb. Technol. 47, 77–83, https://doi.org/10.1016/j.enzmictec.2010.05.005

Xiao K., Song Z.-H., Jiao F.-L., Ke Y.-L., Hu C.-H., 2014. Developmental changes of TGF-β1 and Smads signaling pathway in intestinal adaption of weaned pigs. PLOS ONE 9, e104589,

Xue Z., Yu W., Liu Z., Wu M., Kuo X., Wang J., 2009a. Preparation and antioxidative properties of a rapeseed (*Brassica napus*) protein hydrolysate and three peptide fractions. J. Agric. Food Chem. 57, 5287–5293, https://doi.org/10.1021/jf900860v

Xue Z., Yu W., Wu M., Wang J., 2009b. In vivo antitumor and antioxidative effects of a rapeseed meal protein hydrolysate on an S180 tumor-bearing murine model. Biosci. Biotechnol. Biochem. 73, 2412–2415, https://doi.org/10.1271/bbb.90374

Yamada E.A., Sgarbieri V.C., 2005. Yeast (*Saccharomyces cerevisiae*) protein concentrate: preparation, chemical composition, and nutritional and functional properties. J. Agric. Food Chem. 53, 3931–3936, https://doi.org/10.1021/jf0400821

Yang H., Wu F., Long L., Li T., Liao P., Liu H., Yin Y., 2016. Effects of yeast products on the intestinal morphology, barrier function, cytokine expression, and antioxidant system of weaned piglets. J. Zhejiang Univ. Sci. B 17, 752–762, https://doi.org/10.1631/jzus.B1500192

Yoshida Y., Umeno A., Akazawa Y., Shichiri M., Murotomi K., Horie M., 2015. Chemistry of lipid peroxidation products and their use as biomarkers in early detection of diseases. J. Oleo Sci. 64, 347–356, https://doi.org/10.5650/jos.ess14281

Yuan L., Chang J., Yin Q., Lu M., Di Y., Wang P., Wang Z., Wang E., Lu F., 2017. Fermented soybean meal improves the growth performance, nutrient digestibility, and microbial flora in piglets. Anim. Nutr. 3, 19–24, https://doi.org/10.1016/j.aninu.2016.11.003

Zhou Y. Guan X., Zhu W., Liu Z., Yu H., Wang H., 2014. Capsaicin inhibits *Porphyromonas gingivalis* growth, biofilm formation, gingivomucosal inflammatory cytokine secretion, and in vitro osteoclastogenesis. EJCMID 33, 211–219, https://doi.org/10.1007/s10096-013-1947-0

Zhu J., Mingxing G., Ruili Z. et al., 2017. Effects of soybean meal fermented by *L. plantarum*, *B. subtilis* and *S. cerevisieae* on growth, immune function and intestinal morphology in weaned piglets. Microb. Cell Factories 16, 191, 1–10, https://doi.org/10.1186/s12934-017-0809-3

Zou T., Yang J., Guo X., He Q., Wang Z., You J., 2021. Dietary seaweed-derived polysaccharides improve growth performance of weaned pigs through maintaining intestinal barrier function and modulating gut microbial populations. J. Anim. Sci. Biotechnol. 12, 28, 1–12, https://doi.org/10.1186/s40104-021-00552-8