



ENHANCING NUTRIENT DIGESTIBILITY AND ANTIOXIDANT EFFICACY IN PIGLETS: THE IMPACT OF FERMENTED RAPESEED MEAL SUPPLEMENTATION ON BIOCHEMICAL PARAMETERS AND OXIDATIVE STRESS MARKERS*

Anna Czech¹*, Katarzyna Woś¹*, Siemowit Muszyński², Ewa Tomaszewska³

¹Department of Biochemistry and Toxicology, Faculty of Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, 20-950 Lublin, Poland

²Department of Biophysics, Faculty of Environmental Biology, University of Life Sciences in Lublin, 20-950 Lublin, Poland

³Department of Animal Physiology, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, 20-950 Lublin, Poland

*Corresponding authors: katarzyna.wos00@wp.pl; anna.czech@up.lublin.pl

Abstract

This study aimed to investigate the effects of varying levels of dried fermented rapeseed meal (FRSM) on the nutrient digestibility and antioxidant efficacy in piglets. The experiment was conducted on 300 piglets, starting at 18 days of age. The primary experimental factor was the inclusion of FRSM in the diet, substituting mainly for post-extraction soybean meal and wheat. Two control groups were established: a positive control (PC) supplemented with 2500 ppm zinc oxide, and a negative control (NC) without additives. Additionally, four experimental groups were designated based on FRSM inclusion levels: FR-8 (8% FRSM), FR-12 (12% FRSM), FR-15 (15% FRSM), and FR-25 (25% FRSM). Results indicated a positive impact on nutrient absorption and redox processes, characterized by reduced efficiency of lipid peroxidation products such as malondialdehyde and lipid hydroperoxide in both blood and organs like the liver and intestines. From this perspective, the recommended inclusion of fermented rapeseed meal in piglet feed appears to be within the range of 8–12%.

Key words: fermented rapeseed meal, piglets, digestibility, oxidative markers, blood, tissues

Post-extraction rapeseed meal, a by-product of rapeseed oil extraction, is a valuable source of raw protein (30–42%) and is widely used in the feeding of farm animals due to its market availability (Gołębiowska et al., 2022). However, the presence of anti-nutritional factors (ANFs), such as glucosinolates, tannins, non-starch polysaccharides, and phytic compounds, significantly limits its use, particularly in monogastric animals (Wlazło et al., 2022; Czech et al., 2023; Winiarska-Mieczan et al., 2023). This issue is especially problematic for gestating sows (Czech et al., 2022), which are prone to metabolic syndrome and oxidative stress (Li et al., 2021) and for piglets, whose digestive tracts are sensitive to antinutritional substances (Zhu et al., 2017; Czech et al., 2021 a, b).

One approach to increasing the nutritional potential of rapeseed meal is subjecting it to fermentation (Koo et al., 2018). Fermentation not only enhances the bioavailability of nutrients in rapeseed meal but also stimulates the breakdown of cell wall structures, releasing and/or

inducing the synthesis of various bioactive compounds, particularly antioxidants (Đorđević et al., 2010). Studies have demonstrated that fermented components in feeds reduce undesirable microflora in the gastrointestinal tract (Scharek-Tedin et al., 2015), leading to improved immune system function, nutrient digestibility, and feed efficiency. The stimulation of immune processes is associated with the presence of bacteria or fungi used for fermentation, akin to the action of probiotics, i.e., beneficial bacteria colonizing the intestines. Their presence is crucial for the immune system, supporting immunologic barrier and neutralizing free radicals, thus protecting cells from oxidative stress (Wang et al., 2022), which is essential for young piglets (Augustyniak et al., 2023). Regulating the immune response and positively impacting antioxidative processes, fermented components may additionally effectively protect young organism from pathogens (Yan et al., 2022), potentially reducing the use of pharmaceuticals or therapeutic doses of zinc oxide.

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While the fermentation process for rapeseed meal appears to be an effective strategy for enhancing its nutritional and health value, and numerous studies have examined the impact of fermented feed components on pig nutrition, feed efficiency, and animal health (Xu et al., 2020; Czech et al., 2023), the optimal proportion of these components in the feed mixture to maximize benefits without significantly affecting production profitability remains unclear.

The hypothesis of the study is that fermented rapeseed meal will improve feed efficiency and animal health in young pigs due to increased nutrient bioavailability, reduced anti-nutritional factors, and enhanced antioxidant effects. It was also hypothesized that due to its immunomodulatory properties, it can be added without the inclusion of additional zinc to feed in diets. The study was conducted to test these hypotheses by analyzing the impact of various levels of dried fermented rapeseed meal (FRSM) on nutrient digestibility and antioxidant efficacy in piglets.

Material and methods

The conducted study was approved by the Local Ethics Committee for Animal Experimentation at the University of Life Sciences in Lublin, under permit number 67/2017 issued on September 28, 2017. The barn conditions complied with recommended zoohygienic standards, and the animals were constantly monitored by a veterinary doctor. The animals were under the constant care of a veterinarian. For euthanasia, the piglets were humanely euthanized through exsanguination following captive bolt stunning, in accordance with Council Regulation (EC) No 1099/2009 of September 24, 2009, on the protection of animals at the time of killing.

Animals and the experimental design

The study was carried out with a cohort of piglets (Landrace and Yorkshire Hybrid [LY] × Duroc and Pietrain Hybrid [DP] crossbreeds), born to 30 sows during their second or third pregnancy. To ensure consistent experimental conditions, piglets were evenly distributed among the sows immediately after parturition. This standardization resulted in litters of 14 piglets, with an equal number of female (gilts) and male (barrows) piglets in each. Subsequently, piglets were ear-tagged for clear identification and assignment to their respective replicate. Next, the sows and their litters were randomly divided into six groups, with each group comprising piglets from five sows. The experiment commenced when the piglets were 18 days old (10 days before the planned weaning date) and weighed on average 5.29 kg (Satessa et al., 2020), introducing them at the litter level to their respective experimental diets.

The positive control group (the PC group) of piglets was given a standard pre-starter mix supplemented with 2500 ppm zinc oxide. The negative control group (the NC group) received a standard pre-starter mix without zinc

oxide. The piglets in the FR-8, FR-12, FR-15, and FR-20 groups were fed a mix similar to the NC group, but with wheat and soybean meal partially replaced by dried fermented rapeseed meal (FRSM) at levels of 8%, 12%, 15%, and 20% dry matter, respectively. The fermentation process was previously described by Satessa et al. (2020) and detailed analyzed composition of nutritional value, mineral profile and amino acid profile of FRSM are presented in Czech et al. (2023). Diet formulation was carried out using Agrosoft® (Tørring, Denmark) software, adhering to the Danish nutritional guidelines for piglets (Tybirk, 2015). The pre-starter diet was formulated to be iso-protein and iso-energy across treatments, despite variations in end products, as detailed in Table 1 (Satessa et al., 2020).

Piglets remained with their sows until day 28 of life, after which they were weaned. A total of 50 piglets from each group (10 selected from each litter based on matched body weight and sex, resulting in 25 gilts and 25 barrows in each group) were then transferred to a single pen per treatment replicate in the weaner unit. They continued on the same dietary treatment, based on the pre-weaning diet. The experiment lasted until the piglets reached 41 days old and an approximate weight of 8 kg. The mean values of body weight did not differ between groups (PC: 8.34 kg; NC: 7.78 kg; FR-8: 8.96 kg; FR-12: 9.18 kg; FR-15: 8.79 kg; FR-20: 9.01 kg) (Satessa et al., 2020).

Collection of feces and analysis of digestibility indices

To calculate the nutrient digestibility coefficient, the acid insoluble ash (AIA) method was used, with silicon dioxide (SiO₂) added as a marker to the feed mixtures (2 g/kg) before granulation (Brestenský et al., 2017; Prawirodigo et al., 2021). For the analysis, *n* = 6 randomly selected animals from each group were randomly selected, separated, and were fed the marked feed. Feces were collected seven days before slaughter in the morning hours (6–9 AM) from each pig for the analysis of the digestibility indices of basic nutrients (Klimiuk et al., 2023). Fecal samples were collected over five consecutive days and stored in a refrigerator. Samples from each day were weighed and dried to determine the drying coefficient. Concurrently, feed samples were collected. Both feed and feces were analyzed for dry matter (DM), crude protein (CP), crude fat (CF), and crude fiber (C_{fiber}) using standard AOAC (2016) procedures (930.15, 990.03, 920.39, 978.10, respectively). The concentration of the marker was determined using a gravimetric technique (McCarthy et al., 1974). The content of nitrogen-free extract (NfE) was also calculated. The apparent total tract digestibility (ATTD, %) of the nutrients was calculated using the formula:

$$ATTD = (1 - (N_i \cdot M_d) / (N_d \cdot M_i)) \times 100\%$$

where *N_d* is the concentration of a given nutrient in the diet, *N_i* is the concentration of the nutrient in the feces, *M_d* is the concentration of the marker (SiO₂) in the diet, and *M_i* is the concentration of the marker in the feces (all values expressed in g/kg DM).

Table 1. Basal components and nutritional values of the diets^A

Item	NC	PC	FR-8	FR-12	FR-15	FR-25
Fermented rapeseed meal	0	0	8	12	15	25
Barley	35	35	35	35	35	35
Wheat	33.69	33.24	29.69	26.97	26.08	19.36
Soybean meal	13	13.2	3.9	1.5	0.9	0
Potato protein	2.5	2.5	2.5	2.5	2.5	2.5
Fishmeal	3	3	3	3	3	1.4
Whey powder	3	3	4.25	4.25	4.25	4.25
Whole milk powder	5	5	5	5	5	5
Fermented soybean meal	0.0	0.0	0.0	2.5	1.5	0.0
Vegetable oil	0.7	0.7	4.25	2.87	2.93	3.7
L-Lys (98.5%)	0.74	0.73	0.7	0.76	0.76	0.3
DL-Met (99%)	0.22	0.22	0.21	0.2	0.2	0.2
L-Thr (93.5%)	0.3	0.3	0.3	0.29	0.29	0.3
L-Trp (98%)	0.12	0.12	0.15	0.15	0.15	0.16
Monocalcium phosphate	0.91	0.92	1.02	0.94	0.38	0.78
Sodium chloride	0.25	0.25	0.31	0.35	0.34	0.33
Calcium formate	0.4	0.4	0.7	0.7	0.7	0.7
Sodium bicarbonate	0.15	0.15	0.0	0.0	0.0	0.0
Iron fumarate (31%)	0.25	0.25	0.25	0.25	0.25	0.25
Piger dry aroma	0.2	0.2	0.2	0.2	0.2	0.2
EP premix	0.5	0.5	0.5	0.5	0.5	0.5
Sucram	0.07	0.07	0.07	0.07	0.07	0.07
Medicinal ZnO	0.0	0.25	0.0	0.0	0.0	0.0
Calculated values						
ME (MJ/kg)	13.9	13.4	14.2	14.1	13.5	13.9
calcium (g/kg)	6.24	6.32	7.35	7.47	7.56	7.69
phosphorus (g/kg)	6.20	6.21	6.60	6.60	6.60	6.61
Analyzed values ^A						
dry matter (g/kg)	87.96	88.33	87.87	87.99	88.42	88.07
crude ash (g/kg)	5.69	6.01	5.72	5.88	6.08	5.87
crude protein (g/kg)	19.13	19.08	18.66	18.62	18.49	18.64
crude fat (g/kg)	5.64	5.66	6.89	7.01	6.97	7.37
crude fiber (g/kg)	4.01	3.99	3.38	3.62	3.79	4.32

NC – negative control.

PC – positive control (0.25% ZnO) = PC.

The ingredients 2.5% mixed cakes, 2.5% sugar beet pulp, 2.50% chicory, 1.0% dry apple, 2.50% Nuklospray® E50 provided in pre-starter diets for NC and PC.

0.25% Arbocel® and 0.10% Globigen® Jump Start in pre-starter for NC; 0.5% microbial protein, 0.03% xylanase, 0.03% Ronozyme® VP, 0.1% Mycofix® Plus, 0.5% Tetracid® dry acid, 0.1% ProPen H, 0.2% Acidomatrix™ and 12000 IU beta-xylanase provided in diets for NC and PC.

^ABased on the data and analyzed reported previously (Satessa et al., 2020).

Samples collection

On postnatal day 41, $n = 6$ piglets (three gilts and three barrows) from each treatment group were randomly selected. The selection included one piglet from each of the five replicate litters, identified by ear tags, and an additional piglet selected randomly from the remaining piglets to maintain an equal number of gilts and barrows. These selected piglets were then transported to a commercial slaughter facility for euthanasia. Blood for biochemical analysis was drawn into heparinized tubes

to prevent clotting. The samples were then centrifuged at $1400 \times g$ for 10 minutes at 4°C to separate the plasma, which was aliquoted and stored at -20°C for later analysis. Immediately following exsanguination, the abdominal cavity was opened, and liver samples were collected from the right lobe. Simultaneously, segments of the ileum, each approximately 2 cm in length, were excised and rinsed thoroughly with saline to remove any intestinal content. All tissue samples were harvested within 10 minutes of euthanasia to preserve biological markers.

The collected ileum and liver samples were immediately placed in storage tubes, snap-frozen in liquid nitrogen, and then stored at -86°C until further analysis.

Biochemical blood plasma parameters

Blood plasma underwent a series of analyses to determine levels of various biochemical parameters using colorimetric methods with commercial ready-to-use Liquick Cor test kits (Cormay Diagnostics, Lublin, Poland). The assays included the determination of total protein (TP; kit #2-236), albumin (ALB; kit #2-239), uric acid (UA; kit #2-208), urea (UREA; kit #2-206), creatinine (CREAT; kit #2-233), total bilirubin (BIL; kit #5-173), alkaline phosphatase (ALP; kit #1-212), alanine aminotransferase (ALT; kit #1-216), aspartate aminotransferase (AST; kit #1-214), gamma-glutamyltransferase (GGTP; kit #1-224), total cholesterol (CHOL; kit #2-204), triacylglycerols (TG; kit #2-262), and HDL cholesterol (HDL-C; kit #2-053). Analyses were conducted on a Mindray BS-120 automatic biochemistry analyzer (Bio-Medical Electronics, Shenzhen, China), using colorimetric methods. The accuracy of all analyses was confirmed using multiparametric control serum (Alfa Diagnostics, Warsaw, Poland).

Additionally, LDL cholesterol (LDL-C) levels were calculated using formula (Friedewald et al., 1972):

$$\text{LDL-C (mmol/l)} = \text{CHOL} - \text{HDL-C} - \text{TG}/2.2$$

Redox parameters

In the blood plasma and tissues (liver, ileum), lipid peroxidation products, specifically lipid hydroperoxide (LOOH) and malondialdehyde (MDA), were measured. Additionally, the activities of antioxidative enzymes, including catalase (CAT), reduced glutathione (GSH), and superoxide dismutase (SOD), were spectrophotometrically determined following the established procedures detailed in Tomaszewska et al. (2022). Furthermore, in the blood plasma, additional parameters of the antioxidant system, namely the ferric-reducing ability of plasma (FRAP) and vitamin C content, were also measured as described in Czech et al. (2021 a).

Statistical analysis

Data analysis was performed using Statistica software (v. 13.3, TIBCO Software Inc., Palo Alto, CA, USA). The Shapiro–Wilk test was used to verify the normality of data distribution, while Levene’s test was employed to check the equality of variances. A one-way ANOVA, with treatment as the fixed effect and the pig as the experimental unit ($n = 6$), was utilized to analyze the data. Tukey’s HSD test was applied to determine differences between treatment means. On the FRSM treatments (NC, FR-8, FR-12, FR-15, and FR-25), orthogonal polynomial contrasts were employed to evaluate the linear and quadratic effects of varying FRSM levels on specific response variables. A P-value of less than 0.05 was deemed statistically significant for all tests.

Results

The inclusion of dried FRSM in the ZnO-deprived feed mixture of NC led to a restoration of ATTD of C_{fiber} to levels observed in the PC, zinc-supplemented group. The ATTD of NfE was also restored in all FRSM-supplemented groups, except for the group with the lowest dose. Additionally, FRSM significantly enhanced the ATTD of CF across all doses compared to both the PC and NC groups. In contrast, an increase in CP digestibility was noted in all groups supplemented with FRSM, except for the lowest dose group (Table 2). These changes demonstrated similar linear and quadratic contrasts for CF and C_{fiber} ($P < 0.05$ and $P < 0.001$, respectively), and a quadratic contrast for CP ($P < 0.001$).

Table 2. Apparent total tract digestibility (ATTD) coefficients (%) of basic nutrients

Treatment ¹	CP	CF	C_{fiber}	NfE
PC ²	74.46 c	65.14 c	51.97 a	89.02 ab
NC ³	73.57 c	62.64 c	43.27 b	87.09 b
FR-8	76.55 bc	69.14 b	51.94 a	88.62 ab
FR-12	79.79 a	72.03 a	54.51 a	89.31 a
FR-15	77.91 a	71.81 ab	53.37 a	90.06 a
FR-25	77.87 a	73.29 a	54.09 a	90.54 a
SEM ⁴	0.423	0.701	0.819	0.261
P-value				
TRT ⁵	<0.001	<0.001	<0.001	<0.001
FR ⁶	<0.001	<0.001	<0.001	0.001
linear ⁷	0.116	0.038	0.045	0.991
quadratic ⁷	<0.001	<0.001	0.001	0.227

CP – crude protein, CF – crude fat, C_{fiber} – crude fiber, NfE – nitrogen-free extract.

a–c – means with the same letters are statistically the same across all 6 treatments ($P > 0.05$) based on Tukey’s post hoc test.

¹There were a total of 5 dietary treatments. Diets NC–FR-25 were ZnO free diets: with 0 (NC), 8%, 12%, 15%, or 25% FRSM, respectively. A PC (positive control) diet was formulated on the basis of a basic mixture (NC) with 2.5% ZnO.

²PC – positive control.

³NC – negative control.

⁴SEM – standard error of the means.

⁵P-value for overall effect of dietary treatment (diets PC–FR-25).

⁶P-value for FRSM effect (diets NC–FR-25).

⁷Orthogonal polynomial (linear and quadratic) contrasts were performed to test the effect of FRSM.

In terms of blood biochemical parameters, piglets supplemented with 8% and 12% FRSM exhibited a significant decrease in plasma total protein content ($P < 0.001$) compared to both the positive control (PC) and FR-25 groups. This effect of FRSM inclusion can be characterized as both a linear ($P < 0.01$) and quadratic ($P < 0.001$) response (Table 3). Additionally, FRSM supplementation affected the levels of creatinine and uric acid, with the FR-8 group showing significantly higher values than the other experimental groups ($P < 0.001$). The changes in uric acid content also demonstrated a linear ($P < 0.001$) and a weaker quadratic ($P < 0.05$) response to FRSM inclusion.

Table 3. Values of biochemical indicators in piglets' blood plasma

Treatment ¹	TP g/l	ALB g/l	UREA mmol/l	CREAT μmol/l	UA mmol/l	BIL μmol/l
PC ²	79.56 a	49.82	2.78	103.9 b	0.367 b	1.03
NC ³	73.81 ab	43.71	2.74	110.4 b	0.395 b	1.04
FR-8	63.92 b	45.30	2.81	134.9 a	0.496 a	0.967
FR-12	63.25 b	44.78	2.54	102.3 b	0.365 b	0.961
FR-15	69.59 ab	47.96	1.82	107.2 b	0.401 b	0.847
FR-25	78.39 a	45.25	2.69	106.5 b	0.373 b	0.890
SEM ⁴	1.47	0.797	0.112	2.35	0.010	0.035
P-value						
TRT ⁵	<0.001	0.232	0.083	<0.001	<0.001	0.537
FR ⁶	0.002	0.524	0.100	<0.001	<0.001	0.554
linear ⁷	0.001	0.467	0.649	<0.001	<0.001	0.809
quadratic ⁷	<0.001	0.340	0.170	0.181	0.037	0.578

TP – total protein; ALB – albumin; UREA – urea; CREAT – creatine; UA – uric acid; BIL – bilirubin.

a–b; ^{1–7} – see legend Table 2.

Table 4. Activity of selected enzymes in piglets' blood plasma

Treatment ¹	ALP	ALT	AST	LDH	GGTP
PC ²	162.6 b	43.08 a	59.09 bc	888.4 c	70.23 b
NC ³	234.8 a	27.25 c	51.71 c	639.1 d	35.95 d
FR-8	241.7 a	42.11 a	61.59 b	1110.3 b	103.7 a
FR-12	163.8 b	34.32 b	66.10 b	1102.6 b	39.00 d
FR-15	169.5 b	29.85 c	82.15 a	1347.6 a	59.91 c
FR-25	150.2 b	40.65 a	80.84 a	1105.2 b	56.18 c
SEM ⁴	11.80	1.09	2.01	37.77	3.88
P-value					
TRT ⁵	<0.001	<0.001	<0.001	<0.001	<0.001
FR ⁶	<0.001	<0.001	<0.001	<0.001	<0.001
linear ⁷	<0.001	<0.001	0.568	<0.001	<0.001
quadratic ⁷	0.011	0.229	0.012	<0.001	<0.001

ALP – alkaline phosphatase; ALT – alanine aminotransferase; AST – aspartate aminotransferase GGTP – gamma-glutamylotransferase

a–d; ^{1–7} – see legend Table 2.

The ALP activity in the blood plasma of piglets from the groups with 12%, 15%, and 25% FRSM content, as well as the PC group, was lower compared to the negative control (NC) and FR-8 groups ($P < 0.001$) (Table 4). These changes demonstrated both linear ($P < 0.001$) and quadratic ($P < 0.05$) contrasts. The ALT activity in the blood plasma was significantly higher in piglets from the PC, FR-8, and FR-25 groups compared to the other experimental groups. The inclusion of FRSM showed a quadratic response ($P < 0.05$), leading to an increase in AST activity in the blood plasma relative to the NC group. The highest AST activity was observed in the FR-15 and FR-25 groups, which also differed in the degree of increase in AST activity compared to the PC

group. All groups supplemented with FRSM exhibited increased LDH values compared to both the PC and NC groups, with the highest LDH activity observed in the FR-15 group. The effect of FRSM inclusion on LDH can be characterized by strong linear and quadratic responses ($P < 0.001$). The highest GGTP activity was observed in the FR-8 group, followed by the PC group. In contrast, the lowest GGTP values were recorded in the NC and FR-12 groups, with both linear and quadratic contrasts being significant ($P < 0.001$).

In terms of lipid profile (Table 5), there was a significant decrease in the levels of CHOL and HDL-C in the blood plasma of piglets receiving 15% and 25% FRSM in the FR-15 and FR-25 groups, compared to the other

groups. In the FR-12 group, this decrease was observed in comparison to the NC group. These changes displayed similar linear and quadratic responses ($P < 0.001$ for both), and quadratic contrasts ($P < 0.05$ and $P < 0.01$, respectively), with the highest values of CHOL and HDL-C noted in the blood plasma of piglets from the FR-8 and NC groups. A decline in LDL-C levels was seen in piglets from the FR-25 group compared to all other groups. The %HDL-C showed a quadratic effect ($P < 0.001$), with higher values observed in the NC and FR-8 groups than in the other FRSM-supplemented groups. The %HDL-C in the PC group was also higher compared to the FR-15 group. Compared to the PC group, lower levels of TG were noted in the NC and FR-15 groups, and higher lev-

els in the FR-8 group. In the FRSM groups, these changes demonstrated linear ($P < 0.001$) and quadratic ($P < 0.01$) contrasts.

The inclusion of FRSM led to an increase in SOD activity across all doses compared to both the PC and NC groups, with the highest activity noted in the FR-15 and FR-25 groups, followed by the FR-12 group. This effect of FRSM inclusion showed weak linear and strong quadratic contrasts ($P < 0.05$ and $P < 0.001$, respectively) (Table 6). The FR-25 group exhibited the highest CAT activity. Additionally, the CAT activity in the PC and FR-15 groups was higher compared to the FR-8 and FR-12 groups, demonstrating linear and quadratic contrasts ($P < 0.001$ for both).

Table 5. Lipid profile in piglets' blood plasma

Treatment ¹	CHOL mmol/l	HDL-C mmol/l	LDL-C mmol/l	%HDL-C %	TG mmol/l
PC ²	3.22 b	1.82 b	0.825 a	56.82 ab	1.25 b
NC ³	4.02 a	2.55 a	0.996 a	63.49 a	1.04 c
FR-8	3.83 a	2.34 a	0.823 a	60.92 a	1.48 a
FR-12	3.18 b	1.65 b	0.991 a	51.88 b	1.19 bc
FR-15	2.77 c	1.19 c	0.974 a	42.86 c	1.03 c
FR-25	2.21 c	1.10 c	0.564 b	49.73 bc	1.19 bc
SEM ⁴	0.105	0.093	0.042	1.32	0.030
P-value					
TRT ⁵	<0.001	<0.001	0.011	<0.001	<0.001
FR ⁶	<0.001	<0.001	0.004	<0.001	<0.001
linear ⁷	<0.001	<0.001	0.298	0.654	<0.001
quadratic ⁸	0.033	0.002	0.046	<0.001	0.001

CHOL – total cholesterol; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; %HDL-C – percentage of HDL in CHOL content; TG – triacylglycerols.

a–c; ^{1–8} – see legend Table 2.

Table 6. Oxidative stress markers in piglets' blood plasma

Treatment ¹	SOD U/ml	CAT U/ml	FRAP μmol/l	GSH μmol/l	Vitamin C μmol/l	MDA μmol/l	LOOH μmol/l
PC ²	8.51 d	3.46 b	5.44 c	0.848 c	25.99	6.43 c	3.62 b
NC ³	8.02 d	2.76 bc	5.68 c	0.845 c	25.35	7.37 b	4.32 a
FR-8	11.31 c	2.68 c	7.11 b	2.03 a	26.04	4.95 d	2.88 c
FR-12	13.46 b	2.66 c	9.37 a	1.45 b	26.45	3.21 e	3.32 b
FR-15	14.41 a	3.39 b	9.81 a	1.29 b	24.45	7.40 b	3.43 b
FR-25	14.70 a	5.22 a	10.20 a	1.35 b	26.02	9.54 a	3.55 b
SEM ⁴	0.461	0.161	0.346	0.070	0.233	0.347	0.080
P-value							
TRT ⁵	<0.001	<0.001	<0.001	<0.001	0.159	<0.001	<0.001
FR ⁶	<0.001	<0.001	<0.001	<0.001	0.033	<0.001	<0.001
linear ⁷	0.040	<0.001	0.243	<0.001	0.935	<0.001	<0.001
quadratic ⁸	<0.001	<0.001	0.001	<0.001	0.915	<0.001	<0.001

SOD – superoxide dismutase; CAT – catalase; GSH – reduced and oxidized glutathione; FRAP – ferric ion reducing antioxidant plasma; MDA – malondialdehyde; LOOH – lipid hydroperoxide.

a–e; ^{1–8} – see legend Table 2.

FRSM supplementation increased FRAP and GSH activities across all doses compared to both the PC and NC groups. The highest FRAP activity was observed in the FR-12, FR-15, and FR-25 groups (quadratic effect, $P < 0.01$), and the highest GSH activity was seen in the FR-8 group (linear and quadratic effect, $P < 0.001$ for both). When compared to the PC group, a decrease in MDA was observed in the FR-8 and FR-12 groups, while higher values were noted in the NC, FR-15, and FR-25 groups. In the ZnO-deprived groups (the NC and FRSM groups), both linear and quadratic effects were observed ($P < 0.001$ for both), with the lowest MDA value found in the FR-12 group and the highest in the FR-25 group. Among all experimental groups, the highest LOOH value was observed in the NC group, and the lowest in the FR-8 group. No significant effect of dietary treatment on vitamin C levels was noted.

The results of the analysis of oxidative stress markers in the liver and ileum of piglets are shown in Table 7. In the liver, SOD activity in the FR-15 group was comparable to that in the NC group, both of which were significantly lower than in the other groups. The lowest CAT activity was observed in the NC group, and the inclusion of FRSM progressively increased CAT activity (linear contrast, $P < 0.001$). In the FR-15 and FR-25 groups, CAT activity was higher than in the PC group. Similarly, liver GSH activity gradually increased with rising FRSM levels (linear and quadratic effect, $P < 0.001$ for both), and in all FRSM-supplemented groups, it was

significantly higher compared to both NC and PC groups. Conversely, MDA levels in the liver were significantly lower in all FRSM-supplemented groups than in both control groups (linear and quadratic effect, $P < 0.001$ for both). For LOOH, significantly lower values than in the control groups were observed in the FR-12, FR-15, and FR-25 groups.

In the intestinal epithelium of the ileum, SOD and CAT activities in groups fed with FRSM were significantly higher compared to the NC group and were comparable to those observed in the PC group. The lowest GSH levels were noted in both PC and NC groups, and the inclusion of FRSM showed both linear and quadratic effects ($P < 0.001$ for both). Additionally, the inclusion of FRSM resulted in decreased MDA and LOOH levels in the intestinal epithelium of the ileum compared to both PC and NC groups.

Discussion

Post-extraction rapeseed meal is characterized by a high raw protein content (ranging from 30 to 42%), and a high content of essential amino acids, especially sulfur amino acids. It also possesses a favorable lysine to methionine and cysteine ratio (1:0.83), making rapeseed meal a promising component in pig diets, where the ideal lysine to methionine and cysteine ratio should be around 1:0.65–0.7 (Sobotka and Fiedorowicz-Szatkowska, 2021).

Table 7. Oxidative stress markers in the liver and ileum of piglets

Treatment ¹	SOD U/g	CAT U/g	GSH μmol/g	MDA μmol/g	LOOH μmol/g	SOD U/g	CAT U/g	GSH μmol/g	MDA μmol/g	LOOH μmol/g
Liver					Ileum					
PC ²	197.3 a	78.51 c	0.974 d	11.79 a	8.57 a	144.8 ab	59.03 a	0.818 c	13.49 a	9.97 a
NC ³	186.2 b	72.84 d	1.03 d	11.17 a	8.30 a	138.6 b	54.76 b	0.868 c	13.15 a	9.92 a
FR-8	199.2 a	81.97 c	1.34 bc	7.93 b	7.65 ab	149.1 a	59.74 a	1.12 ab	8.47 b	8.33 b
FR-12	196.1 a	80.91 c	1.27 c	6.97 b	7.34 b	148.3 a	59.89 a	1.06 b	7.66 b	8.20 b
FR-15	193.2 b	89.38 b	1.37 b	7.17 b	6.87 b	146.8 a	59.02 a	1.15 ab	7.81 b	7.42 b
FR-25	195.8 a	94.85 a	1.40 a	6.92 b	6.63 b	147.8 a	61.17 a	1.17 a	7.55 b	7.26 b
SEM ⁴	0.851	1.25	0.030	0.355	0.160	0.770	0.447	0.025	0.445	0.212
P-value										
TRT ⁵	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FR ⁶	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
linear ⁷	<0.001	<0.001	0.001	<0.001	0.798	<0.001	0.137	0.001	<0.001	0.142
quadratic ⁸	<0.001	0.700	<0.001	<0.001	0.340	<0.001	0.047	<0.001	<0.001	0.006

SOD – superoxide dismutase; CAT – catalase; GSH – reduced and oxidized glutathione; MDA – malondialdehyde; LOOH – lipid hydroperoxide.

a–d; ^{1–8} – see legend Table 2.

In this study, even the inclusion of 8% FRSM led to an increase in total protein (TP) digestibility. This finding aligns with research by Feng et al. (2007), who noted enhanced trypsin activity in the duodenum and jejunum of 5-week-old piglets following fermented soybean meal consumption. Yan et al. (2022) also reported a 17% increase in crude protein (CP) digestibility in weaned piglets fed with fermented soybean meal. This improvement in protein digestibility could be attributed to the action of microorganisms that break down proteins during fermentation (Adeola et al., 2014). Mukherjee et al. (2016) observed a 5.6% increase in small peptides in the serum of broilers fed a diet with fermented soybean meal. However, in our study, despite enhanced protein digestibility, TP in the piglets' blood serum decreased at lower FRSM inclusions (8 and 12%) and increased gradually with higher doses, but still remained lower than in the control group. The reduced stomach pH, possibly due to the presence of *Lactobacillus*, might stimulate protein hydrolysis, contributing to increased CP digestibility in animals fed a diet with fermented components (Koo et al., 2018). *Lactobacillus*, being a valuable source of peptides and phenols, may increase peptide content and facilitate the breakdown of glucosinolates during FRSM fermentation. The decrease in glucosinolate levels is likely due to the utilization of glucose and sulfur groups by the microorganisms involved in fermentation (Wang et al., 2022). Microbiological fermentation of rapeseed meal (RM) also reduces the dietary fiber content by up to 35.39% (Wang et al., 2022). Given that dietary fiber is known to negatively impact nutrient digestibility (Agyekum and Nyachoti, 2017), the observed increase in crude fiber digestibility in groups fed a diet with FRSM inclusion, particularly at the 12% level, can be explained by *Lactobacillus* enhancing the activity of certain digestive enzymes. This effect potentially improves the digestibility of components in the dietary mixture. Additionally, gut microbiota modulation (increase in *Lactobacillus* and decrease in *E. coli*) positively affects the immune system, strengthening the intestinal barrier, thus further enhancing nutrient digestibility (Lan et al., 2016). Fermentation may also reduce levels of undesirable components like phytic acid, tannins, or certain phenols, thereby improving nutrient availability. The microorganisms involved in the fermentation process can secrete digestive enzymes, contributing to this effect (Wang et al., 2018; Liu et al., 2022).

Previous research has highlighted the effectiveness of FRSM in mitigating oxidative stress in piglets (Sun et al., 2020; Wang et al., 2009). This aspect is critical, particularly during the weaning period when piglets face dietary changes, social adjustments, and varying hygienic conditions, leading to oxidative stress and lipid peroxidation. Malondialdehyde (MDA) serves as a primary indicator of lipid peroxidation and is known for its high mutagenicity. In the present study, the inclusion of FRSM at 8% and 12% led to a notable reduction in blood plasma levels of lipid peroxidation

products, including LOOH and MDA. This finding suggests that these specific FRSM doses are effective in counteracting the damaging effects of reactive oxygen species. Wu et al. (2022) reported similar findings, showing that a 5% FRSM inclusion in broiler diets reduced serum MDA levels by approximately 40%. Chen et al. (2020) observed that adding 0.2% *Lactobacillus delbrueckii* to the diet decreased MDA in the small intestine. Wang et al. (2009) achieved a similar reduction in liver MDA using *L. fermentum*. In the present study, a significant decrease in both MDA and LOOH levels was detected in the liver and intestines of piglets fed diets with various FRSM inclusions.

The enhancement of antioxidant protection can also be ascribed to the fermentation process, which enriches rapeseed meal with bioactive compounds like vitamins, enzymes, polysaccharides, and additional polyphenols. A significant phenolic component in rapeseed meal is sinapine, which accounts for up to 80% of all phenols and is known for its robust antioxidant properties (Liu et al., 2023). The mechanism of polyphenols in antioxidative defense primarily involves scavenging reactive oxygen species, such as hydroxyl and superoxide radicals (Zhao et al., 2021), and chelating transition metal ions like Fe^{+2} , which are known to generate free oxygen radicals (Taranu et al., 2022).

In the present study, the incorporation of FRSM into the diet led to an increase in antioxidant protection, as indicated by the enhanced activity of redox enzymes (SOD, CAT, GSH) in the blood plasma, liver, and epithelium of the ileum. Notably, the most significant elevation in enzyme activity in the blood plasma was observed with the highest FRSM inclusion level, 25%. However, even with the lowest FRSM inclusion level of 8%, the study documented a considerable increase in the activity of these redox enzymes.

In the liver and intestinal epithelium, the highest increase in SOD activity was observed in piglets fed a diet containing 8% FRSM. However, the greatest activities of CAT and GSH were found in the group receiving the highest FRSM inclusion of 25%. Notably, an increase in redox enzyme activity was evident even at the lowest FRSM dose. Wu et al. (2022) reported a 14.5% increase in SOD activity in broiler chickens fed a diet with 5% FRSM compared to a control group fed a corn and soy protein-based feed. Interestingly, the SOD activity in the FRSM-fed groups was slightly lower by 0.7% than in groups fed with raw rapeseed meal (Wu et al., 2022). The enhancement of redox enzyme activities with fermented soybean meal has also been documented in rat studies (Yoon et al., 2014; Huang et al., 2022) and in research involving weaned piglets supplemented with 0.1–0.2% *L. salivarius* (Sun et al., 2020). Wang et al. (2009) found that *L. fermentum* supplementation increased CAT activity in pig livers. Similarly, the inclusion of *Lactobacillus delbrueckii* (0.2%) was shown to enhance GSH activity in pig intestines (Chen et al., 2020). Moreover, Buckley et al. (2018) observed that supplementation with *Lacto-*

bacillus salivarius (0.5–0.2%) not only raised GSH but also SOD and CAT activities.

The presence of *Lactobacillus* in FRSM may offer probiotic benefits, including lactic acid and prebiotic oligosaccharides, which could enhance the body's ability to counter oxidative stress. Oxidative stress, along with dysbiosis of gut microbiota, can contribute to liver diseases. Free radicals, generated by oxidative stress, activate Kupffer cells, leading to the production of pro-inflammatory factors such as tumor necrosis factor- α (TNF- α) and transforming growth factor-beta (TGF- β), exacerbating liver inflammation (Polimeni et al., 2015). The probiotic action of FRSM is conducive to gut health, and its bioactive compounds help mitigate oxidative stress effects, thereby improving liver function (Zhu et al., 2021).

In this study, the inclusion of FRSM in piglet diets was found to benefit liver health, as evidenced by a decrease in ALT activity with just an 8% FRSM inclusion. However, a reduction in ALP activity was observed only in the group fed a diet with 25% FRSM. These findings align with those of Zhu et al. (2021), who observed a nearly 24% decrease in ALT activity in rats fed a high-fat diet supplemented with fermented black barley, compared to a control group. Interestingly, Zhu et al. (2021) also reported a 4.6% decrease in AST activity, which contrasts with the increase in AST activity observed in this study with higher FRSM doses. Additionally, this study noted a decrease in GGTP activity but an increase in LDH activity in the FRSM-supplemented groups. While existing research indicates that fermented products positively impact liver function, further studies are necessary to ascertain the optimal FRSM dosage for piglets that would beneficially influence liver health.

Analyzing the biochemical parameters of the piglets involved in the study revealed several key observations. Firstly, AST activity in the FR-15 and FR-25 groups, along with uric acid levels in all groups, and cholesterol and triglyceride contents – particularly in the NC group – were observed to be higher than reference values for adult pigs (Winnicka, 2021). These anomalies are believed to stem from an excessive intake of easily digestible protein in the piglets' diet. Such nutritional imbalance can lead to conditions like kidney inflammation and liver failure, among other health issues (Tumbleson and Meade, 1966). The presence of high protein levels in feed can induce metabolic stress, potentially impairing the function of vital organs. Furthermore, it is important to consider that dietary crude protein in piglet diets has been shown to influence hepatic lipid metabolism. This influence encompasses various metabolic processes, including lipogenesis, lipolysis, oxidation, and gluconeogenesis in the liver (Liu et al., 2021). Thus, managing protein levels in feed is crucial for maintaining optimal health and metabolic balance in piglets.

Previous studies have shown that incorporating fermented products containing *Lactobacillus* spp. into diets can have a hypocholesterolemic effect, notably reducing

cholesterol and LDL levels in the blood (Sanders, 2000; Liong and Shah, 2005). This effect was also observed in the current study, but it was significant only in animals fed a diet with a 25% inclusion of FRSM. Moreover, it was noted that even an 8% addition of FRSM led to a reduction in triglyceride levels in the blood. This finding aligns with earlier research on piglets where diets supplemented with dried, fermented soybean and/or rapeseed meal were used (Czech et al., 2021 b). However, unlike the Czech et al. (2021 b) study where an increase in HDL was linearly related to the level of fermented component, the current study did not observe such a relationship; an increase in HDL levels was only noted in the FR-8 group, with HDL levels decreasing as the proportion of fermented rapeseed meal increased. The positive influence of fermented products on lipid metabolism is also supported by a study conducted by Nie et al. (2020) on broilers using fermented cottonseed meal. The beneficial effects on lipid metabolism are likely related to the probiotic properties of the feed mixture containing fermented rapeseed meal (Qu et al., 2022). Previous studies have highlighted the role of certain *Lactobacillus* strains in mitigating lipid metabolism disorders. The hypolipidemic properties of these probiotics are linked to their ability to modulate the transcription of genes involved in lipid metabolism (Zhong et al., 2012; Xin et al., 2017). Additionally, changes in gut microbiota following the inclusion of *Lactobacillus*-fermented products in animal diets have been documented. These changes include a decrease in bacteria typically associated with negative effects on lipid metabolism and an increase in beneficial bacteria like *Lactobacillus* and *Bifidobacterium* (Qu et al., 2022). *Lactobacillus* spp. and *Bifidobacterium* are known to secrete hydrolase, which catalyzes the hydrolysis of bile acids. The results of the current study might be linked to this hydrolysis and deconjugation of bile salts induced by lactic acid bacteria, disrupting the reabsorption of bile salts from the small intestine and leading to increased excretion of bile salts in feces. This process necessitates enhanced synthesis of bile acids in the liver, using cholesterol as a precursor. Thus, the deconjugation of bile salts can contribute to the reduction of serum cholesterol levels (Liong and Shah, 2005; Jazi et al., 2018).

This study has certain limitations. Extending the duration of the experiment could provide a more detailed evaluation of the long-term effects of fermented rapeseed meal on animal health. Different developmental stages of animals might respond differently to fermented rapeseed meal, thus investigating its impact on various age groups could yield more diverse and informative data. Another important aspect is the influence of specific bacterial strains used in the fermentation process. Understanding the role of different bacteria in enhancing the nutritional value and health benefits of fermented products is essential. Further research into the diversity of bacteria involved in fermentation and their specific contributions is critical for a deeper comprehension of the health and nutritional benefits of different microorganisms. Future

studies incorporating these considerations will enhance our understanding of the broader, long-term effects of rapeseed meal fermentation, and contribute significantly to the knowledge of how this process affects animal health.

Conclusion

The present experiment demonstrates the substantial benefits of including fermented post-extraction rapeseed meal in piglet diets. The use of fermented rapeseed meal at concentrations of 8–25% has been shown to enhance nutrient absorption efficiency and positively impact redox processes, evidenced by the decreased levels of lipid peroxidation products such as MDA and LOOH in the blood, liver, and intestines. From this perspective, the optimal inclusion level of fermented rapeseed meal in piglet feed appears to be between 8–12%.

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