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Biochemical and haematological blood parameters of sows and piglets fed a diet with a dried fermented rapeseed meal

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Abstract

Animal health and welfare can be assessed using biochemical and haematological markers of the blood. The values of these parameters depend in part on the quantity and quality of feed ingredients, i.e. feed protein and feed additives. The aim of the study was to determine the effect of including fermented rapeseed meal (FRSM) in dry feeding system on haematological and biochemical blood parameters of sows and piglets. The experimental material comprised 30 primiparous gilts and 30 multiparous sows after their second lactation. They were randomly divided into two groups of equal size – control and experimental. The animals in control groups C_G (15 gilts) and C_S (15 sows) received a standard diet for pregnant or lactating sows, depending on the reproductive period. Experimental groups E_G and E_S were 15 gilts and 15 multiparous sows, respectively, receiving feed with a 4% share of FRSM in place of soybean meal up to 100 d of gestation. In addition, from 100 d of gestation to 7 d of lactation, the sows in these groups received feed with a 9% share of FRSM, and then again a diet with a 4% share of FRSM until the end of lactation. Blood samples were taken from 6 animals from each group in two periods: at 100 days of pregnancy (late pregnancy) and at 27 days of lactation (late

lactation). Blood from piglets was taken at 27 days of age (before weaning), from two piglets from each sow (one gilt and one barrow), taking into account the average body weight in the litter. Haematological parameters: Ht, Hb and RBC were determined in whole blood. The plasma content of minerals, activity of selected enzymes and biochemical parameters of sows, gilts and piglets were determined. The diet containing fermented rapeseed meal, fed to pregnant and lactating sows, increased the level of Ht and Hb and RBC content and mineral content (phosphorus, calcium and iron) in the plasma. This effect was mainly observed in primiparous sows. The inclusion of FRSM in the diet of sows reduced the plasma content of total cholesterol and triacylglycerols in sows and piglets, as well as liver enzyme activity, particularly AST in piglets. The use of fermented rapeseed meal in sow diet resulted in better use of mineral compounds, improvement of production effects and health parameters of sow and piglet blood.

Key words: sows, piglets, fermentation, fermented rapeseed meal, blood

Determination of biochemical and haematological blood parameters is one means of assessing animal health and welfare (Marco-Ramell et al., 2016). The values of these parameters depend in part on the quantity and quality of feed ingredients. The basic protein components of pig feed are soybean meal, rapeseed meal and leguminous seeds. In preparing diets for monogastric livestock, attempts are made to find GMO-free protein feedstuffs (Florou-Paneri et al., 2014). One of these can be rapeseed extraction meal. However, its use in feeding pigs is limited by the presence of many anti-nutritional factors (ANFs), such as glucosinolates, phytates or tannins (Dingyuan and Jianjun 2007; Gu et al., 2010). These compounds can impair nutrient absorption, induce metabolic disorders, and modify endocrine or reproductive functions (Choct et al., 2010; Woyengo et al., 2017). Monogastric animals are susceptible to the adverse effects of these substances (Gu et al., 2010; Nega, 2018). Therefore, efforts are made to reduce ANFs in compound feeds, or even eliminate them altogether, by cultivating new varieties or through technological treatments. One way to reduce ANFs in rapeseed meal is to subject it to the action of microbes (fermentation) whose enzymes can effectively hydrolyse proteins and break down anti-nutrients (Canibe and Jensen, 2012). In addition, fermentation, by causing cell wall degradation, reduces the level of non-starch sugars and induces synthesis of various bioactive compounds, such as enzymes, organic acids and bacteriocins (El-Batal and Abdel Karem, 2001; Vig and Walia, 2001; Hu et al., 2010; Shi et al., 2015; Chi and Cho, 2016; Su et al., 2018). Enteric pathogens are also reduced during fermentation, which improves the safety of feed and food (Canibe and Jensen, 2012; Cheng et al., 2019).

The feed fermentation process has previously been used in preparing liquid diets (Canibe et al., 2007; Canibe and Jensen, 2012; Missotten et al., 2015). In recent years, attention has been drawn to the use of fermented feedstuffs in dry feed (Navarro et al., 2017). This applies in particular to rapeseed extraction meal, although there are also reports of fermentation of the seeds of pulses: lupine, peas, or soybean meal (Nega, 2018). Thus far there are only a few studies, carried out mainly on poultry and pigs, which indicate that it can be used to replace some portion of GMO and non-GMO soybean meal (Chiang et al., 2010; Fazhi et al., 2011). Fermented feeds, owing to the presence of lactic acid, other short-chain organic acids, and probiotic bacteria, as well as degradation of phytate complexes, improve the absorption of amino acids and minerals, including phosphorus, calcium, zinc, copper and iron. This can modify metabolic processes in the body, including the haematological, biochemical and mineral profile of the blood (Kim et al., 2007; Shi et al., 2016). Fermented rapeseed meal improves production efficiency by increasing the overall use of nutrients, including minerals (Grela et al. 2019), and therefore a change in the hematological, biochemical and mineral profile of the blood can be expected.

The aim of the study was to determine the effect of including fermented rapeseed meal in dry feeding system on haematological and biochemical blood parameters of sows and piglets.

Material and methods

The experimental procedures used throughout this study were approved by the II Local Ethics Committee on Animal Experimentation of University of Life Sciences in Lublin, Poland, (Resolution No. 21/2016).

Experimental design

The experimental material comprised 60 Yorkshire sows mated with Danish Landrace boars. These included 30 primiparous gilts and 30 multiparous sows after their second lactation. They were randomly divided into two groups of equal size – control and experimental. The animals in control groups C_G (gilts) and C_S (sows) received a standard diet for pregnant or lactating sows, depending on the reproductive period. Experimental groups E_G and E_S were gilts and multiparous sows, respectively, receiving feed with a 4% share of fermented rapeseed meal (FRSM) in place of soybean meal up from 28 d to 100 d of gestation. In addition, from 100 d of gestation to 7 d of lactation, the sows in these groups received feed with a 9% share of FRSM, and then again a diet with a 4% share of FRSM until the end of lactation. During gestation, the sows stayed in pens with five animals apiece (group feeding), and from two weeks before parturition until weaning they were housed in individual stalls (individual feeding). FRSM was obtained from European Protein AS (Bække, Denmark).

Animal diets

The sows were fed dry compound feed in accordance with NRC (2012). Compound feeds for the control groups contained cereal meal (wheat, triticale, barley and oat), extracted soybean meal, rapeseed oil, mineral feeds (monocalcium phosphate, salt and limestone), an acidifier, and a mineral-vitamin premix. The mineral-vitamin premix contained the following in 1 kg: vitamin A 620,000 IU, vitamin D3 80,000 IU, vitamin E 800 mg, vitamin K3 100 mg, vitamin B1 80 mg, vitamin B2 280 mg, vitamin B6 200 mg, vitamin B9 60 mg , vitamin B12 1000 mcg, vitamin H 10,000 mcg, vitamin PP 800 mg, vitamin B5 600 mg, choline 15,000 mg, iron 4,000 mg, copper 800 mg, zinc 4000 mg, manganese 2000 mg, selenium 10 mg, iodine 30 mg, L-lysine 30,000 mg, L-methionine 10,000 mg, and threonine 10,000 mg. The acidifier contained the following in 1 kg: orthophosphoric acid, (E 338) 320 g, citric acid (E 330) 110 g, fumaric acid (E2 97) 50 g, propionic acid (E 280) 45 g, formic acid (E 236) 45 g, and silicon dioxide as a carrier – 430 g. Gestation diets (2.5 kg per day) were supplied twice a day (08:00 and 18:00). On d 108 of gestation, sows were transported to farrowing stalls and individually fed. After farrowing, all sows received the experimental lactation diet (Table 1). The lactation diet was supplied three times a day (08:00, 12:00 and 18:00), starting at 2.0 kg/day and increasing by 0.5 kg/day during the first week. Afterwards, sows had free access to the diet until they were weaned on d 28 of lactation. Sows were provided ad libitum access to water during the entire experimental trial. The nutrients and ANFs of the diets for the animals in the control and experimental groups are given in Table 1.The results of all analyses are presented in Grela et al. (2019).

The piglets fed on the sows' milk and additionally received a prestarter diet. Prestarter feed (13.6 MJ EM, 215 g CP, total lysine 13.5 g) was available to the piglets ad libitum from d 10 of age and continued for 1 week after weaning. From the second week, the piglets were fed a starter diet (13.5 MJ EM, 207 g CP, total lysine 11.5 g). The diets for piglets did not contain FRSM, and their nutritional value and vitamin and mineral content were in accordance with NRC (2012) for piglets weighing 5–7 kg.

Experimental procedures

Blood samples were taken from 6 sows from each group in two periods: at 100 days of pregnancy (late pregnancy) and at 27 days of lactation (late lactation). Blood was always collected from the same animals. Blood from piglets was taken at 27 days of age (before weaning), from two piglets from each sow (one gilt and one barrow), taking into account the average body weight in the litter.

For twelve hours before blood collection the sows had no access to feed and the piglets had no access to sows' milk. Blood was collected into 10 ml heparin tubes from the jugular vein under veterinary supervision. Haematocrit (Ht), haemoglobin content (Hb) and erythrocyte count (RBC) were determined in whole blood using an ABACUS-Vet analyser (Hungary).

The biochemical parameters and mineral content were determined in blood plasma, which was obtained by centrifuging whole blood at 3000 g for 10 min. Analyses were performed 3–4 h after the blood was collected. The blood was stored at 4°C.

Test kits developed by Cormay (Lublin, Poland) were used to determine the content of total protein (TP - Liquick Cor-TOTAL PROTEIN 60, catalogue No 2-236, Poland), albumin (ALB - Liquick Cor-Albumin 60, catalogue No 2-238, Poland), glucose (GLU - Liquick Cor-GLUCOSE 60, catalogue No 2-201, Poland), triglycerides (TG - Liquick Cor-TG 30, catalogue No 2-262, Poland), total cholesterol (TCH - Liquick Cor-CHOL 60, catalogue No 2-204, Poland), high-density lipoprotein cholesterol (HDL - Liquick Cor-HDL, catalogue No 2-053, Poland), and total iron-binding capacity (TIBC - Liquick Cor-TIBC 500, catalogue No 2-306, Poland).

The percentage of HDL in relation to total cholesterol (% HDL) was calculated as well.

Ready-to-use test kits were used to determine the activity of selected enzymes: alanine aminotransferase (ALT - Liquick Cor-ALAT 60, catalogue No 1-216, Poland), aspartate aminotransferase (AST - Liquick Cor-ASAT 60, catalogue No 1-214, Poland), alkaline phosphatase (ALP - Liquick Cor-ALP 60, catalogue No 1-212, Poland), lactate dehydrogenase (LDH - Liquick Cor-LDH 30, catalogue No 1-239, Poland), and gamma-glutamyltransferase (GGT - Liquick Cor-GGT 60, catalogue No 1-224, Poland). The plasma content of phosphorus (P - Liquick Cor-PHOSPHORUS 30, catalogue No 3-243, Poland), calcium (Ca - Liquick Cor-CALCIUM ARSENOZO 60, catalogue No 3-255, Poland), magnesium (Mg - Liquick Cor-MG, catalogue No 3-229, Poland), iron (Fe - Liquick Cor-FERRUM, catalogue No 3-258, Poland), zinc (Zn – BioMaxima catalogue No 99 28 14, Poland) and copper (Cu - BioMaxima catalogue No 99 33 05, Poland) was determined by the colorimetric method.

Statistical analysis

The data on compound feeds and blood parameters of the sows and piglets were subjected to statistical evaluation by two-factor analysis with interaction, taking into account the following factors:

$y_{ijk} = C_i + F_j + (C \text{ x } F)_{ij} + e_{ijk}$

where: y_{ijk} – observations; C_i – effect of reproductive cycle (primiparous gilts or multiparous sows); F_j – effect of animal feeding group (control or experimental – the effect of dried fermented rapeseed meal); $(C \times F)_{ij}$ – effect of interaction between reproductive cycle and diet. Statistical significance between treatments was based on $P < 0.05$ and $P < 0.01$. Analyses were performed in the GLM procedure of SAS 9.4 (SAS Institute, Cary NC).

Results

The compound feeds for the experimental animals had a higher content of lactic acid and a reduced amount of phytate phosphorus (Table 1).

Table 1. Content of nutrients and bioactive substances in 1 kg of feed (Grela et al., 2019)

Ingredients	FRSM[1]	Early pregnancy[*]		Mid-pregnancy/ late lactation[**]		Late pregnancy/early lactation[***]	
		C_{EP}	E_{EP}	C_{MPL}	E_{MPL}	C_{LPL}	E_{LPL}
Analysed:							
Dry matter, g	882.7	883	882	889	888	887	885
Crude ash, g	78.9	50.6	49.8	52.4	51.9	52.3	51.8
Crude protein, g	291.8	150.3	150.1	170.4	171.2	171.3	172.1
Ether extract, g	31.7	32.2	32.3	27.2	27.3	27.3	27.5
Crude fibre, g	91.5	73.5	74.4	48.5	50.2	48.9	51.1

Total phosphorus, g	9.09	5.23	5.19	5.63	5.67	5.71	5.69
Phytate phosphorus, g	5.73	3.14[a]	2.31[b]	3.79[a]	1.94[b]	3.77[a]	2.01[b]
Calcium, g	8.05	7.45	7.43	8.62	8.63	8.65	8.71
Sodium, g	2.26	2.04	2.03	1.98	1.97	1.91	1.93
Zinc, mg	66.98	142.5	144.3	148.7	149.2	143.8	144.5
Copper, mg	6.67	18.61	18.42	20.30	20.43	20.22	20.11
Iron, mg	149.2	145.8	149.2	165.3	166.2	164.5	166.1
Glucosinolates, μmol g^{-1}	6.37	0.001	0.002	0.001	0.002	0.001[a]	0.004[b]
Tannins, g kg^{-1}	4.76	2.19	2.34	2.21	2.35	2.17	2.39
Lactic acid, mmol l^{-1}	50.4	17.1[b]	51.2[a]	16.9[b]	59.9[a]	16.6[b]	84.3[a]
Calculated:							
Metabolizable energy, MJ	12.27	12.3	12.3	12.6	12.6	12.6	12.7

*sows up to 84 days of pregnancy; **sows from 85 to 100 days of pregnancy and from 8 to 28 days of lactation; *** sows from 101 to 114 days of pregnancy and up to day 7 of lactation, C_{EP} = control sows in early pregnancy; E_{EP} = experimental sows in early pregnancy; C_{MPL} = control sows in mid-pregnancy and late lactation; E_{MPL} = experimental sows in mid-pregnancy and late lactation; C_{LPL} = control sows in late pregnancy and early lactation; E_{LPL} – experimental sows in late pregnancy and early lactation.

[1]FRSM – fermented rapeseed meal

a, b - different letters between C_{EP} *vs* E_{EP}; C_{MPL} *vs* E_{MPL} and C_{LPL} *vs* E_{LPL} denote statistically significant differences at P≤0.05.

The addition of FSRM to the diet of pregnant sows significantly influenced the plasma content of phosphorus, copper and iron (Table 2). Significantly higher content of these elements was found in both primiparous (EG) and multiparous (ES) sows relative to the corresponding control groups (CG and CS). The number of reproductive cycle (primiparous vs multiparous – R) was also found to influence the level of copper (higher in primiparous sows) and zinc (higher in multiparous sows) (Table 2). Plasma content of phosphorus, magnesium, copper and iron was significantly increased in the primiparous and multiparous sows during lactation in the group of experimental animals. The addition of FRSM increased the zinc content in the plasma of multiparous sows. Analysis of the influence of the reproductive cycle (primiparous versus multiparous sows – lactation period) revealed significant relationships for the plasma content of zinc (higher in primiparous sows) and iron (higher in multiparous sows).

In the piglets born of primiparous sows (EG), a significant increase was noted in the plasma content of all analysed minerals, except magnesium, in comparison to controls (CG). In the case of piglets from multiparous sows (ES), such a relationship was found for calcium and iron (Table 2).

Table 2. Mineral content in the blood plasma of sows and piglets

Minerals	Gilts		SEM	Sows		SEM	F	R	F x R
	C_G	E_G		C_S	E_S			P_{value}	
Late pregnancy (n = 6)									
Phosphorus; mmol l^{-1}	1.42^B	1.70^A	0.036	1.38^x	1.88^y	0.030	<0.001	0.095	0.403
Calcium; mmol l^{-1}	2.04	2.22	0.048	2.09	2.21	0.041	0.118	0.842	0.747

Magnesium; mmol l^{-1}	1.05	1.09	0.022	1.06	1.12	0.022	0.242	0.609	0.741
Copper; µmol l^{-1}	26.19^{B}	32.47^{A}	0.999	23.37^{x}	28.09^{y}	0.950	0.001	0.025	0.605
Zinc; µmol l^{-1}	9.12	9.79	0.333	11.12	11.02	0.255	0.499	<0.001	0.357
Iron; µmol l^{-1}	15.38^{B}	21.06^{A}	1.042	13.88^{X}	19.18^{Y}	0.736	<0.001	0.114	0.857
Late lactation (n = 6)									
Phosphorus; mmol l^{-1}	1.87^{B}	2.31^{A}	0.042	1.76^{X}	2.24^{Y}	0.055	<0.001	0.276	0.798
Calcium; mmol l^{-1}	2.72	2.80	0.056	2.72	2.94	0.041	0.133	0.480	0.497
Magnesium; mmol l^{-1}	1.04^{B}	1.27^{A}	0.017	1.03^{X}	1.28^{Y}	0.022	<0.001	0.890	0.783
Copper; µmol l^{-1}	12.66^{B}	16.15^{A}	0.634	14.65^{X}	18.31^{Y}	0.535	0.023	0.074	0.084
Zinc; µmol l^{-1}	18.03	18.10	0.762	14.18^{X}	17.78^{Y}	0.455	<0.001	0.001	0.874
Iron; µmol l^{-1}	18.78^{B}	25.43^{A}	0.801	23.54^{x}	29.17^{y}	0.743	<0.001	0.002	0.681
Piglets (n = 12)									
Phosphorus; mmol l^{-1}	1.97^{B}	2.42^{A}	0.033	2.31	2.58	0.061	<0.001	0.009	0.300
Calcium; mmol l^{-1}	2.58^{b}	3.12^{a}	0.041	2.53^{X}	3.04^{Y}	0.047	<0.001	0.317	0.103
Magnesium; mmol l^{-1}	1.30	1.39	0.029	1.48	1.45	0.039	0.674	0.098	0.377

Copper; µmol l^{-1}	20.66^b	26.91^a	0.701	25.45	25.25	0.976	0.124	0.419	0.103
Zinc; µmol l^{-1}	7.87^B	10.29^A	0.287	8.17	9.43	0.311	0.002	0.601	0.279
Iron; µmol l^{-1}	27.81^b	35.33^a	1.092	28.02^x	37.51^y	1.143	<0.001	0.539	0.613

Legend: F = effect of FRSM; R = effect of reproductive cycle; FxR = interaction between experimental factor (FRSM) and reproductive cycle; C_G = control gilts; E_G = gilts receiving diet with FSRM; C_S = control sows; E_S = sows receiving diet with FSRM.

a, b – values in rows (gilts) with different letters are significantly different at $P \leq 0.05$: x, y – values in rows (sows) with different letters are significantly different at $P \leq 0.05$: A, B – values in rows (gilts) with different letters are significantly different at $P \leq 0.01$: X, Y – values in rows (sows) with different letters are significantly different at $P \leq 0.01$

SEM - standard error of the mean refers to result to parameters in blood plasma of gilts and sows

The inclusion of fermented rapeseed meal (FRSM) significantly increased red blood cell parameters (Ht, Hb and RBC) in the primiparous gilts during lactation and in their piglets relative to the control group (C_G). Analysis of the effect of the reproductive cycle (primiparous vs multiparous – R) revealed a higher erythrocyte count in the blood of pregnant gilts, a higher Ht in lactating gilts, and lower Hb content in lactating gilts (Table 3). Piglets from primiparous gilts also had higher Ht and Hb values than piglets from multiparous sows.

Table 3. Red blood cell counts of sows and piglets

Trait	Gilts		SEM	Sows		SEM	F	R	F x R
	C_G	E_G		C_S	Es			P_{value}	
Late pregnancy (n = 6)									

Ht; %	38.77^{b}	41.12^{a}	0.387	39.38	39.11	0.464	0.263	0.449	0.163
Hb; mmol l^{-1}	7.01^{b}	7.88^{a}	0.055	6.83	6.81	0.089	0.187	0.657	0.245
RBC; 10^{12} l^{-1}	6.91^{B}	8.01^{A}	0.087	6.44	6.63	0.122	0.020	<0.001	0.265
Late lactation (n = 6)									
Ht; %	36.23^{b}	39.17^{a}	0.567	34.05	35.99	0.737	0.010	0.004	0.570
Hb; mmol l^{-1}	4.56^{B}	5.98^{A}	0.100	6.79	6.88	0.138	0.019	0.006	0.098
RBC; 10^{12} l^{-1}	6.23^{b}	6.96^{a}	0.121	6.31	6.74	0.165	0.003	0.900	0.774
Piglets (n = 12)									
Ht; %	36.24^{b}	39.96^{a}	0.359	34.05	35.99	0.546	0.009	0.004	0.570
Hb; mmol l^{-1}	6.92^{b}	7.89^{a}	1.023	6.79	6.88	1.311	0.017	0.008	0.098
RBC; 10^{12} l^{-1}	6.03^{b}	6.76^{a}	0.077	6.31	6.94	0.090	0.004	0.860	0.784

Legend: see Table 2

RBC - red blood cell count; Hb - haemoglobin content; Ht - haematocrit

Pregnant sows receiving a diet with FRSM (groups E_G and E_S) had significantly higher plasma concentrations of total protein (TP) and albumin (ALB) compared to sows from the control groups (C_G and C_S), and in the case of primiparous sows (E_G), higher iron-binding capacity (TIBC) as well. In lactating sows, such a relationship was recorded only in the case of plasma ALB content in multiparous sows. Piglets from both primiparous and multiparous sows receiving a diet with FSRM had significantly higher concentrations of Glu, TP, ALB and TIBC than piglets from the corresponding control groups (C_G and C_S). The reproductive parity (primiparous vs multiparous – R) influenced the plasma concentrations of TP, ALB and TIBC in pregnant sows, TIBC in lactating sows, and TP in piglets from these sows. These parameters were higher in multiparous sows as well as in piglets from these sows (Table 4).

Table 4. Content of biochemical parameters of blood plasma of sows and piglets

Trait	Gilts		SEM	Sows		SEM	F	R	F x R
	C_G	E_G		C_S	Es			P_{value}	
Late pregnancy (n = 6)									
Glu; mmol l^{-1}	3.25	3.47	0.089	3.41	3.90	0.077	0.094	0.111	0.613
TP; g l^{-1}	57.00^B	66.48^A	1.236	67.70^Y	75.40^X	1.362	<0.001	<0.001	0.355
ALB; g l^{-1}	40.86^b	44.08^a	0.710	41.87^y	44.86^x	0.601	0.045	<0.001	0.569
TIBC; µmol l^{-1}	41.06^b	49.51^a	2.000	59.56	62.42	2.22	0.049	<0.001	0.316
Late lactation (n = 6)									
Glu; mmol l^{-1}	3.67	3.68	0.141	3.06	3.48	0.155	0.066	0.685	0.492
TP; g l^{-1}	63.84	61.78	1.730	60.91	61.81	1.398	0.082	0.144	0.072
ALB; g l^{-1}	42.02	44.23	1.298	40.04^y	44.74^x	0.867	0.424	0.139	0.458
TIBC; µmol l^{-1}	40.96	44.51	1.444	59.42	63.56	1.278	0.057	0.015	0.770
Piglets (n = 12)									
Glu; mmol l^{-1}	6.71^b	7.71^a	0.125	6.69^y	7.35^x	0.123	<0.001	0.396	0.429
TP; g l^{-1}	44.73^b	52.85^a	1.351	52.97^y	57.36^x	1.158	0.002	0.002	0.315
ALB; g l^{-1}	40.18^b	45.34^a	0.888	44.96^y	48.37^x	0.786	0.035	0.252	0.497
TIBC; µmol l^{-1}	21.86^b	26.31^a	0.691	23.50^y	29.06^x	0.765	<0.001	0.103	0.675

Legend: see Table 2

Glu - glucose; TP - total protein; ALB - albumin; TIBC - total iron-binding capacity

In the pregnant primiparous and multiparous sows receiving a diet with FSRM (E_G and E_S), there was a significant decreased in the plasma concentration of total cholesterol (CHOL), while the HDL fraction increased relative to the control groups (C_G and C_S) (Table 5). In multiparous sows, a significant reduction in the concentration of triacylglycerols was noted. In piglets from sows receiving FRSM (E_G and E_S), there was also a significant reduction in the level of CHOL and its HDL fractions relative to the control groups, and in the case of piglets from multiparous sows (E_S), in triacylglycerols as well. Analysis of the reproductive cycle (primiparous vs multiparous sows) indicated a significantly higher share of HDL and a lower level of triacylglycerols in primiparous sows during pregnancy (Table 5).

Table 5. Lipid parameters in the plasma of sows and piglets

Trait	Gilts		SEM	Sows		SEM	F	R	F x R
	C_G	E_G		C_S	Es			P_{value}	
Late pregnancy (n = 6)									
CHOL; mmol l^{-1}	2.08^A	1.77^B	0.254	2.02^a	1.82^b	0.034	<0.001	0.917	0.419
HDL; mmol l^{-1}	1.14	1.18	0.061	1.00	1.10	0.055	0.301	0.090	0.652
% HDL	55.08^b	66.60^a	1.531	49.48^B	60.62^A	1.611	<0.001	0.038	0.941
TG; mmol l^{-1}	0.841	0.780	0.039	1.01^a	0.793^b	0.027	0.002	0.040	0.057
Late lactation (n = 6)									
CHOL; mmol l^{-1}	1.84^A	1.38^B	0.030	1.50	1.41	0.064	0.010	0.127	0.068
HDL; mmol l^{-1}	0.951	1.04	0.039	0.75^B	1.01^A	0.030	0.002	0.031	0.089
% HDL	52.07^B	75.20^A	3.041	51.22^B	72.01^A	2.709	<0.001	0.523	0.710
TG; mmol l^{-1}	0.470^A	0.340^B	0.013	0.401^a	0.251^b	0.017	<0.001	0.007	0.705

Piglets (n = 12)									
CHOL; mmol l^{-1}	2.01^{a}	1.61^{b}	0.053	2.11^{B}	1.54^{A}	0.062	<0.001	0.914	0.453
HDL; mmol l^{-1}	0.958	0.949	0.052	1.22^{a}	0.895^{b}	0.036	0.046	0.205	0.058
% HDL	47.96^{B}	58.97^{A}	1.002	57.91	59.06	1.300	0.026	0.073	0.011
TG; mmol l^{-1}	0.917	1.06	0.056	1.30^{a}	0.974^{b}	0.035	0.014	0.112	0.324

Legend: see Table 2

CHOL - total cholesterol; HDL - high density lipoprotein; TG – triacylglycerols

The inclusion of FSRM in the diet significantly influenced the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in pregnant sows, alkaline phosphatase (ALP) and AST in lactating sows, and all analysed enzymes (ALP, ALT, AST, LDH and GGT) in piglets (Table 6). In both primiparous and multiparous sows receiving a diet with FRSM (groups E_G and E_S) during pregnancy, AST activity was significantly lower than in the control groups (C_G and C_S). Similar trends were observed in the case of ALT in group E_G. ALP activity in lactating sows from groups E_G and E_S was significantly higher than in the corresponding control groups C_G and C_S. The reverse relationship was observed for AST activity, which was significantly lower in the plasma of sows from groups E_G and E_S than for C_G and C_S, respectively. Piglets from sows receiving a diet with FRSM had significantly higher plasma activity of ALP and ALT, but significantly lower activity of AST and lactate dehydrogenase (LDH) than piglets from the control groups (C_G and C_S). The reproductive cycle (primiparous vs multiparous sows) influenced the activity of ALP (higher in primiparous sows), ALT and AST (higher in multiparous sows) during pregnancy. During lactation, ALT activity was higher in primiparous sows, while AST activity was higher in multiparous sows (Table 6).

Table 6. Activity of selected enzymes (U l^{-1}) in the plasma of sows and piglets

Trait	Gilts		SEM	Sows		SEM	F	R	F x R
	C_G	E_G		C_S	Es			P_{value}	
Late pregnancy(n = 6)									
ALP	175.67	169.99	7.258	125.43	130.52	6.450	0.975	<0.001	0.569
ALT	41.31^a	33.44^b	1.098	62.58	60.61	2.992	0.038	<0.001	0.197
AST	32.45^A	24.80^B	1.333	44.31^X	38.86^Y	1.432	<0.001	<0.001	0.330
LDH	1994.2	2210.6	58.12	2064.9	1857.1	69.83	0.973	0.274	0.107
GGT	37.73	44.34	3.698	60.88	57.42	3.543	0.661	0.238	0.099
Late lactation (n = 6)									
ALP	152.4^b	180.5^a	6.489	130.4^Y	186.9^X	5.112	<0.001	0.194	0.024
ALT	29.42	24.89	2.399	23.74	20.55	1.209	0.085	0.029	0.755
AST	26.44^A	16.17^B	2.018	44.33^X	27.60^Y	2.004	<0.001	<0.001	0.053
LDH	759.4	791.1	18.26	848.6	815.1	16.76	0.094	0.771	0.071
GGT	51.83	52.14	3.061	61.78	52.24	3.632	0.434	0.395	0.404
Piglets (n = 12)									
ALP	126.83^B	161.11^A	3.511	151.4^y	183.7^x	4.035	<0.001	<0.001	0.877
ALT	22.21^b	26.90^a	0.648	21.50^y	28.86^x	0.509	<0.001	0.681	0.360
AST	53.50^a	45.29^b	1.141	46.78^x	39.80^y	1.325	0.006	0.022	0.807
LDH	2137.3^A	1365.5^B	77.35	2046.4^X	1286.5^Y	80.11	<0.001	0.361	0.949
GGT	26.01	34.83	2.941	31.18	35.78	3.875	0.008	0.206	0.378

Legend: see Table 2

ALP - alkaline phosphatase; ALT - alanine aminotransferase; AST - aspartate aminotransferase;

LDH - lactate dehydrogenase; GGT - glutamyltransferase

Discussion

One method of improving the suitability of rapeseed meal for feeding monogastric animals is fermentation (Jakobsen et al., 2015). Research conducted by Grela et al. (2019) shows that the addition of a fermented rapeseed component to diets significantly improves production parameters, mainly in primiparous gilts, which leads to increased litter size (c.a. 17 %) and litter weight (c.a. 8.8 %) at 28 days of age. Fermented rapeseed meal improved production efficiency by increasing the overall use of nutrients, increased the immunoglobulin concentration in colostrum (IgG by approx. 31 % in multiparous and 40 % in primiparous sows and IgM by about 2 % in multiparous and 23 % in primiparous sows), and therefore a change in the hematological, biochemical and mineral profile of blood can be expected. It also helped to improve the digestibility of crude protein (by up to 2.6 %), fat and crude fibre and positively affected the gut microbiota of sows (especially gilts during late pregnancy). Fermentation of rapeseed meal is an effective way to reduce anti-nutrients (phytate phosphate and glucosinolates) and to increase the level of lactic acid in compound feeds, as well as to stimulate the immune system, which improves the health of piglets, reduce diarrhoea severity (by 72 % in piglets born to multiparous sows and by 145 % in piglets born to primiparous sows) and mortality (by 9.4 % in piglets born to multiparous sows and by 141 % in piglets born to primiparous sows) (Grela et al., 2019).

Research by Jakobsen et al. (2015) and Shi et al. (2015) has shown that fermentation improves the availability not only of nutrients but of minerals as well, which is confirmed in the present study. The increase in the plasma content of phosphorus in pregnant and lactating sows whose feed contained FRSM (E_G and E_S), as well as in their piglets, was probably the result of increased availability of phosphorus, which has also been demonstrated by El-Batal and Karem (2001). The content of non-phytate phosphorus in the feeds with a fermented component was over 1.5 times higher than in the control feeds (C_{EP}, C_{MPL} and C_{LPL}). Research by Pedersen et al. (2007) and Stein et al. (2009) has shown an increase in phosphorus bioavailability in animals receiving a diet based on fermented maize. It should be noted that the values of these minerals were within the ranges reported by Friendship and Henry (1996), Winnicka (2011) and Klem et al. (2010). In addition to phosphorus, the piglets from sows receiving feed with FRSM also had higher plasma content of calcium. The reason for this effect, as in the case of phosphorus, was probably the increase in phytase synthesized by microorganisms during fermentation (Shi et al., 2016). The presence of phytase caused phosphorus and calcium to be released from chelates, thus increasing their availability (Czech and Grela, 2004; Czech et al., 2010; Iqbal et al., 2012). At the same time, it should be noted that the fermented component used in the research was subjected to a drying process and that the technology of this treatment does not affect the composition of microorganisms produced during fermentation. Liesegang et al. (2005) have also reported an increase in the content of minerals in the plasma of sows receiving a diet containing microbial phytase. Research by Czech and Grela (2004) has confirmed the effectiveness of phytase in the diet of pregnant and lactating sows, as evidenced by increased content of minerals, particularly phosphorus and calcium, in the colostrum and milk.

In parallel with the increase in the plasma content of phosphorus and calcium in sows whose feed included the fermented component, as well as in piglets from these sows, there was an increase in iron concentration, and in the case of pregnant and lactating sows, an increase in copper as well. In addition, other extracellular degradation enzymes of microbial origin (with the exception of phytase), such as cellulase, hemicellulase, glycoside hydrolase, protease and peroxidase, as well as small peptides and free amino acids, could also improve the availability of mineral elements (Shi et al., 2016). According to Juanpere et al. (2005), enzymes hydrolysing non-starch polysaccharide (NSP) fractions may contribute to better conditions for the action of microbial phytase in the gut. The available literature contains many studies confirming the role of phytase and NSP-degrading enzymes in improving the absorption of nutrients and minerals from feed (Jongbloed et al., 2000; Kim et al., 2005).

The better availability of iron in the animals receiving a diet with a fermented component is confirmed by the slightly elevated (and in the case of piglets significantly elevated) total iron-binding capacity of the plasma (TIBC). The increase in iron and copper content in animals receiving diets with FRSM corresponded with the increase in blood haematological parameters. The haematological parameters in all experimental groups were consistent with the values reported by Czech et al. (2018). This effect was particularly evident in primiparous sows (E_G) during both pregnancy and lactation and in piglets from these sows.

Gilts are characterized by less fat share in the carcass, increased metabolism associated with the increase of body weight and accumulation of reserves for fetal development and milk production [Yang et al., 2009]. Thus, the use of mixtures containing the fermented component, by improving the availability of nutrients and minerals, as well as lowering the gastrointestinal pH, thereby increasing the amount of lactic acid bacteria and reducing the amount of intestinal pathogens, had a positive effect on the digestibility of nutrients and stimulation of erythropoiesis [Smiricky Tjardes et al., 2003].

The plasma content of iron in the sows and piglets in all experimental groups was similar to that reported by Friendship and Henry (1996) for analogous production groups.

Czech (2007) have shown an increase in iron concentration in the plasma of sows receiving phytase and a multi-enzyme preparation. A study by Kim et al. (2005) also confirmed an interaction between microbial phytase and NSP-degrading 1,4-β-xylanase (4000 U g^{-1}) synthesized by microorganisms produced in the fermentation process, which increases the digestibility of calcium and phosphorus.

The increase in total protein content, and in particular albumin, in the plasma of pregnant sows receiving FRSM in their diet, as well as in piglets from sows whose feed contained FRSM, can be explained by increased availability of nutrients (Kim et al., 2007). It should be emphasized that the improved biochemical and haematological parameters in piglets from sows receiving FRSM were reflected in decreased diarrhoea and higher weight gains in these piglets (Grela et al., 2019). This is due in part to the presence of phytase, which improves the availability of protein from feed (Guggenbuhl et al., 2007), as well as to the presence of lactic acid, which supports phytase activity and nutrient absorption (Missotten et al., 2015).

These animals also had a significantly higher proportion of the HDL cholesterol fraction. This may indicate a positive effect of fermented feed components and stimulation of lipid metabolism, which is closely linked to normal thyroid function (Webb, 2010). According to Hung et al. (2008), this reaction could also be associated with intake of large quantities of lactic acid bacteria with fermented feeds, which can modify lipid metabolism (Yonejima et al., 2013). The presence of fermented components influences the absorption of lipids through the intestinal walls and subsequently their metabolism (Jensen, 1998).

The presence of microbial phytase (microorganisms produced in the fermentation process), by improving the availability of phosphorus and calcium, can be presumed to have a significant impact on the ossification process, which is highly favourable in pregnant or lactating sows as well as in young piglets. This is indicated in the present study by the increase in markers of bone metabolism, such as alkaline phosphatase activity (lactating sows and piglets), osteocalcin and procollagen peptides, as well as by the results of physicochemical bone tests (Swaminathan, 2001; Tomaszewska et al., 2019). In addition, the reduced content of anti-nutritional substances may decrease the activity of liver enzymes, mainly AST, which was observed in piglets born to sows fed a diet with FRSM. An increased AST/ALT ratio is associated with progressive impairment of liver function, due in part to the presence of glucosinolates in the diet of pigs (Giannini et al., 1999). Recent studies have shown that fermentation of rapeseed meal can improve growth performance and influence biochemical parameters in poultry (Chiang et al., 2010; Webb, 2010). The activity of the enzymes tested (ALP, AST, ALT) in the plasma of the sows and piglets in all experimental groups was similar to the values reported by Friendship and Henry (1996) for analogous production groups.

Conclusions

The diet containing fermented rapeseed meal, fed to pregnant and lactating sows, increased the Ht value, Hb content and RBC count as well as mineral content (phosphorus, calcium and iron) in the plasma. This effect was mainly observed in primiparous sows. The inclusion of FRSM in the diet of sows reduced the plasma content of total cholesterol and triacylglycerols in sows and piglets, as well as liver enzyme activity, particularly AST in piglets. The use of fermented rapeseed meal in sow diet resulted in better use of mineral compounds, improvement of production effects and health parameters of sow and piglet blood.

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Author Contributions

A.C. collected the data, performed laboratory and data analyses and co-wrote the manuscript. E.R.G. experimental design, feed components, performed data analyses and co-wrote the manuscript. M.K. collected data and co-wrote the manuscript and performed data analyses, S.K. performed laboratory analyses, All authors read the manuscript and approved of its content.

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Conflicts of Interest

I have read the manuscript and I hereby affirm that the content of this manuscript or a major portion thereof has not been published in a refereed journal, and it is not being submitted fully or partially for publication elsewhere. The manuscript has been read and approved by all listed authors. There is no conflict of interest.