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Physiological responses of rabbits fed with diets containing rapeseed meal, white lupine and pea seeds as soybean meal substitutes

Resposta fisiológica de coelhos alimentados com dietas que contêm farelo de canola, semente de tremoço branco e de ervilha como substitutos do farelo de soja extraído por meio de solventes

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ABSTRACT

In recent years, a trend has emerged to eliminate soybean meal (SBM) from rabbit diets. It was hypothesized that a dietary mixture composed of rapeseed meal (RSM), white lupine seeds (WLS) and pea seeds (PS) could be a substitute for SBM in diets for growing rabbits without compromising their growth and physiological parameters. To verify this hypothesis, selected parameters describing the growth rate of rabbits, their blood and slaughter parameters and caecum function were analyzed. The experiment was performed on ninety HYPLUS rabbits. Control group (C) animals were fed a diet containing 15% SBM. The diet administered to the first experimental group (E1) contained 7.5% SBM, 5.0% RSM, 4.0% WLS and 3.0% PS. In the diet fed to the second experimental group (E2), SBM was completely replaced with RSM, WLS and PS. The substitution did not exert a significant negative effect on the growth rate of rabbits, selected morphological and biochemical blood parameters or carcass characteristics. The comparable growth parameters of control group rabbits and rabbits fed diets containing SBM substitutes could be partially attributed to beneficial changes in the enzymatic activity of caecal microbiota, which have a positive influence on fermentation processes in the lower gastrointestinal tract. The results of this study indicate that SBM can be completely replaced with a mixture of RSM, WLS and PS in growing rabbit diets.

Index terms: Rabbit feeding; growth performance; blood parameters, gut function.

RESUMO

Nos últimos anos, observa-se uma tendência de eliminar farelo de soja (SBM) da dieta dos coelhos. Uma hipótese foi feita em que a mistura composta por farelo de canola (RSM), semente de tremoço branco (WLS) e semente de ervilha (PS) pode substituir o SBM em dietas de coelhos sem comprometer o crescimento e parâmetros fisiológicos. Para verificar esta hipótese, analisou-se determinados parâmetros que descrevem o tempo de crescimento dos coelhos, os parâmetros de sangue e de abate, assim como a função do contra-ângulo. O ensaio foi realizado em noventa coelhos HYPLUS. Os animais do grupo de controle (C) foram alimentados com dieta contendo 15% de SBM. A dieta no primeiro grupo experimental (E1) foi composta por 7,5% de SBM, 5,0% de RSM, 4,0% de WLS e 3,0% de PS. Na dieta do segundo grupo experimental (E2), o SBM foi totalmente substituído por RSM, WLS e PS. A substituição não teve impacto negativo relevante sobre o crescimento dos coelhos, em parâmetros morfológicos e bioquímicos de sangue, nem sobre características das carcaças. Os parâmetros comparáveis de crescimento de coelhos do grupo de controle e de coelhos do grupo alimentado com dieta contendo substitutos do SBM, podem ser parcialmente atribuídos a mudanças favoráveis na atividade enzimática da microflora intestinal, que têm impacto positivo sobre processos de fermentação no trato gastrointestinal inferior. Os resultados destes ensaios indicam que, na dieta de coelhos em crescimento, o SBM pode ser inteiramente substituído pela mistura de RSM, WLS e PS.

Termos para indexação: Alimentação de coelhos; parâmetros de crescimento; parâmetros sanguíneos; funcionamento do sistema digestivo.

INTRODUCTION

In recent years in the countries where soybean is not grown, studies have been conducted to completely or partially replace soybean meal in rabbit diets in order to use other locally grown protein sources instead of imported soybeans (Attia; El-Deek; Osman, 1998; Attia; Al-Harthi; El-Deek, 2003). Therefore, soybean meal has been replaced with other high-protein components such as rapeseed by-products (Gasmi-Boubaker et al., 2007; Strychalski et al., 2014; Gugolek et al., 2015) or legume seeds, mostly peas - *Pisum sativum* (Lounaouci-Ouyed; Berchiche; Gidenne,

2014; Zwolinski et al., 2017) and white lupine - *Lupinus albus* (Volek; Marounek, 2011; Volek; Volkova; Marounek, 2013; Volek et al., 2014; Uhlirova et al., 2015; Zwolinski et al., 2017). The results of most studies were positive. However, it should be noted that the rearing period is relatively short in rabbits, and the potentially adverse effects of nutrition may not fully manifest themselves. This is an important consideration since legume plants and their by-products contain numerous antinutritional factors including tannins, antitrypsin, hemagglutinins, α-galactosidases, alkaloids and mycotoxins (Kasprowicz; Frankiewicz, 2003; Chilomer et al., 2010). Researchers are divided in their opinions about the influence of legumes, particularly lupins on rabbit performance, but Kelly, Cheeke and Patton (1990) analyzed the efficacy of white lupine in rabbit nutrition and found that the animals were tolerant of lupine alkaloids.

The influence of diet composition can be evaluated based on not only performance parameters but also hematological and blood biochemical or gastrointestinal function indicators. Etim et al. (2014) reported that different diets fed to rabbits exerted different effects on hematology parameters. Gbore and Olatunbosun (2010) demonstrated that analyses of hematology and serum biochemistry in rabbits can also be used to detect the presence of anti-nutritional dietary factors. Our previous studies indicated that feeding has a significant effect on gastrointestinal tract parameters in rabbits (Strychalski et al., 2014; Gugolek et al., 2015).

It was hypothesized that a dietary mixture composed of rapeseed meal, white lupine and pea seeds and other conventional feed ingredients could be effective substitutes for SBM-based diets in growing rabbits without compromising their growth and physiological parameters. To verify this hypothesis, selected parameters describing the growth rate of rabbits, their blood and slaughter parameters and caecum function were analyzed.

MATERIAL AND METHODS

The present study is part of a long-term research project investigating the replacement of SBM in diets fed to rabbits of various breeds with other local protein sources, conducted at the University of Warmia and Mazury in Olsztyn, Poland. Control group (C) animals were fed a diet containing 15% soybean meal (SBM). The diet administered to the first experimental group (E1) contained 7.5% SBM, 5.0% rapeseed meal (RSM), 4.0% white lupine seeds (WLS) var. Wat and 3.0% pea seeds (PS) var. Mecenas. The seeds were not dehulled. In the diet fed to the second experimental group (E2), SBM was completely replaced with RSM, WLS and PS. Chemical composition and energy value of SBM, RSM, WLS and PS are shown in Table 1. The ingredients, chemical composition and energy content of diets are presented in Table 2. All diets were characterized by similar levels of protein and energy, and their nutritional value corresponded to the requirements of growing meat-type rabbits.

Animal protocol

The experimental animals were HYPLUS line rabbits reared on a farm located in central Poland. Growth parameters were measured in 90 animals (45 females and 45 males) selected from 15 litters (six rabbits from each litter) and randomly allocated to three groups. The experiment was conducted in May-July. The animals were 35 days old and had average body weight of 952.6 ± 4.57 g (mean ± SEM) when the experiment began, and they were 84 days old when it ended.

Table 1: Chemical composition and energy content of soyabean meal, rapessed meal, white lupin seeds and pea seeds.

	Soyabean meal	Rapessed meal	White lupin seeds	Pea seeds
Chemical composition (%)				
Dry matter	90.1	91.0	88.8	88.1
Crude ash	6.3	7.2	4.1	3.4
Total protein	45.2	34.6	41.1	22.3
Crude fat	1.8	3.6	5.0	0.9
Crude fibre	4.6	15.2	13.5	5.7
N-free extracts	32.2	30.4	25.1	55.8
Energy content (MJ/kg)				
Digestible energy	12.5	9.9	10.3	12.3

Table 2: Ingredients, chemical composition and energy content of feed mixtures.

	Group		
	C	E1	E2
Composition of feed mixtures (%)			
Soybean meal	15.0	7.5	-
Rapeseed meal	-	5.0	10.0
White lupine seed	-	4.0	8.0
Pea seed	-	3.0	6.0
Barley	14.5	12.5	10.5
Wheat	6.0	7.5	9.0
Corn	16.0	12.5	9.0
Dried alfalfa	23.0	23.0	23.0
Wheat bran	11.0	11.0	11.0
ARBOCEL *	6.0	5.5	5.0
Beet molasses	2.0	2.0	2.0
Skimmed milk powder	2.0	2.0	2.0
Dried brewer's yeast	1.0	1.0	1.0
Calcium carbonate	1.0	1.0	1.0
Dicalcium phosphate	1.0	1.0	1.0
Mineral-vitamin premix **	1.0	1.0	1.0
NaCl	0.5	0.5	0.5
Chemical composition of feed mixtures (%)			
Dry matter	90.7	91.0	90.6
Crude ash	6.6	6.7	6.2
Organic matter	84.1	84.3	84.4
Total protein	17.4	18.0	18.0
Crude fat	2.3	2.9	3.4
Crude fibre	12.4	13.1	14.7
N-free extracts	52.0	50.3	48.3
Lysine	0.78	0.80	0.82
Methionine + cystine	0.54	0.56	0.58
Threonine	0.70	0.74	0.77
Thryptophan	0.16	0.16	0.15
Energy content (MJ/kg)			
Digestible energy	10.6	10.4	10.1

* - crude fibre concentrate.

** - Composition Mineral-vitamin premix 1 kg: vit. A - 3 500 000 IU, vit. D_3 - 200 000 IU., vit. E - 28 000 mg, vit. K_3 - 200 mg, vit. B_1 - 1 500 mg, vit. B_2 - 2 800 mg, vit. B_6 - 2 800 mg, vit. B_{12} - 20 000 mcg, folic acid- 200 mg, niacin - 10 000 mg, biotin - 200 000 mcg, calcium pantothenate - 7 000 mg, choline - 30 000 mg, Fe - 17 000 mg, Zn - 2 000 mg, Mn - 1 000 mg, Cu (copper sulfate x 5H2O, 24,5%) - 800 mg, Co - 1 000 mg, I - 100 mg, methionine - 150 g, Ca - 150 g, P - 100 g.

The experiment was performed in an indoor experimental facility at the University of Warmia and Mazury in Olsztyn (NE Poland). Rabbits were kept in wire-mesh flat-deck cages with the size of 0.5 × 0.6 × 0.4 m (two animals per cage). They had *ad libitum* access to feed served once a day via automatic feeders and water from nipple drinkers. The pellets had average length of 12 mm and average diameter of 4 mm. The animals received no medications or other substances during the experiment. They were housed under standard conditions with a temperature of 18-20 °C, relative air humidity of 60-75%, intensive ventilation of rooms, and regulated photoperiod (16 h of light and 8 h of dark).

The animal handling protocol and the number of animals used in this study were consistent with regulations of the Local Institutional Animal Care and Use Committee (Olsztyn, Poland), and the study was carried out in accordance with EU Directive 2010/63/EU for animal experiments (OJEU, 2010).

Experimental procedures

During the performance trial, the rabbits were weighed on an electronic scale with an accuracy of 1 g, and their live weight was determined at 35, 56 and 84 days. Average daily body weight gains and feed conversion ratio (body weight gain / feed intake) were also calculated.

Seven days before the end of the experiment, 5 males and 5 females were randomly selected from each group. Blood samples were collected from the ear vein. Blood was sampled to heparinized 2.5 mL test tubes under the supervision of a veterinarian. The following hematology and biochemistry parameters determinations were made: total white blood cell count (WBC), lymphocyte percentage (LYM), medium-sized cell percentage (MID), granulocyte percentage (GRA), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDWc), platelet count (PLT), platelet percentage (PCT), mean platelet volume (MPV), platelet distribution width (PDWc), total protein (TP), glucose (Glu), cholesterol (Chol), triglycerides (TG), free fatty acids (FFA), glutamic pyruvic transferase (GPT), glutamic oxoloacetic transaminase (GOT), gamma-glutamyl transferase (GGTP).

After the feeding experiment, the animals were fasted for 24 h and sacrificed according to the standard guidelines for euthanizing experimental animals. Carcasses were skinned and eviscerated. The head was dissected along the occipital joint; the forepart was dissected between the 7th and 8th thoracic vertebra and the loin was dissected

between the 6th and 7th lumbar vertebra. The hind part with the perisacral area and hind legs was the remaining part of the carcass after dissection (Daszkiewicz et. al., 2012). The following slaughter performance data were collected: pre-slaughter weight, hot carcass weight with the head, hot carcass weight without the head, the weights of head, liver, kidneys, heart, lungs, skin and legs, cold carcass weight without the head, inedible portion weight. Dressing percentage (DP) was calculated according to the formula provided by Blasco and Ouhayoun (1993): DP = carcass weight without the head / slaughter weight × 100%. The weight of the most valuable cuts, forepart, loin and hind part, in the carcass was also calculated.

Selected parameters of the gastrointestinal tracts of four males and four females from each group were analyzed immediately after slaughter. After laparotomy, the caecum was removed and digesta was weighed. Samples of fresh digesta were used for analysis of volatile fatty acids (VFAs) and the activity of bacterial enzymes.

Analytical methods

The content of individual nutrients in diets was determined with standard methods (AOAC International, 2006). The digestible energy (DE) of the diet was calculated as described by Bovera et al. (2012): DE = 13.68 - 0.2472 x CF, where DE is the apparent DE (MJ/kg) and CF is the crude fibre (% as-fed).

Selected hematological parameters, mentioned above, were measured in whole blood samples with the use of the ABACUS Jr VET Analyzer (DIATRON MI PLC, Budapest, Hungary), while biochemical parameters - Akcent 200 (Cormay).

The concentrations of VFAs in samples of caecal digesta were analyzed in a gas chromatograph (Shimadzu GC-2010, Kyoto, Japan). The samples (0.2 g) were mixed with 0.2 mL of formic acid, diluted with deionized water, and centrifuged at 7211g for 10 min. The supernatant was transferred to a vial and then loaded onto a capillary column (SGE BP21, 30 m × 0.53 mm using an on-column injector. The initial oven temperature was 85 °C; it was raised to 180 °C in steps of 8 °C/min and maintained at this level for 3 min. The temperature of the flame ionization detector and the injection port was 180 °C and 85 °C, respectively. The volume of the sample for gas chromatography was 1 µL. The concentrations of putrefactive VFAs (PVFAs) in caecal digesta were calculated as the sum of iso-butyrate, iso-valerate, and valerate. All VFA analyses were performed in duplicate. Pure acetic, propionic, butyric, iso-butyric, iso-valeric and valeric acids were obtained from Sigma Co. (Poznań, Poland), and they were combined to create a standard plot and to calculate the amount of each acid. This additional set of pure acids was included in each GC run at five sampling intervals to maintain calibration. The VFA pool was calculated as the sum of VFA concentrations in the digesta and the relative weight of caecal digesta.

In addition to VFA analysis, caecal fermentation processes were analyzed based on the activity of selected bacterial enzymes (α-and β-glucosidase, α- and β-galactosidase, β-glucuronidase, α-arabinopyranosidase, α-arabinofuranosidase, β-xylosidase and β-cellobiosidase), which was measured by the rate of release of ρ-nitrophenol or o-nitrophenol from the respective nitrophenylglucosides, according to a previously described method (Juskiewicz et al., 2014). To determine the total activity of the above enzymes, a sample of caecal digesta diluted in 100mM phosphate buffer (pH 7.0) was mechanically disrupted by vortexing with glass beads (212-300 µm in diameter; four periods of 1 min each, with 1 min cooling intervals on ice) in the FastPrep®-24 homogenizer (MP Biomedicals, Santa Ana, Ca, US). The resulting sample was centrifuged at 7211 g for 15 min at 4 ºC. A reaction mixture containing 0.3 ml of the substrate solution (5 mM) and 0.2 ml of the caecal sample was prepared. Incubation was carried out at 37 ºC, and ρ-nitrophenol was quantified at 400 nm (o-nitrophenol was quantified at 420 nm) after the addition of 2.5 ml of 0.25 M-cold sodium carbonate. Enzyme activity was expressed as µmol product formed per hour per g of digesta. The enzyme activity was expressed in µmol of the product (PNP or ONP, ρ-nitrophenol or o-nitrophenol, respectively) formed per hour per gram of digesta. The respective calculation formulas were derived based on the model curves for PNP and ONP (PNP or ONP standard solution in a 100mM phosphate buffer, pH 7.0, 40 mg/L). All analyses were performed in duplicate.

Statistical analyses

Data are expressed as means ± standard error of the mean (SEM). Pen was considered the experimental unit. The results were processed statistically using least squares means in GLM procedures. For comparison of data, the $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha_i\beta_j + \varepsilon_{ijk}$ model was used, where µ is the general mean, α_i is the effect of diet, β_j is the effect of sex, $\alpha_i\beta_j$ is the interaction effect between diet and sex, and ε_{ijk} is the random error. Significance of differences among groups was determined with the Duncan multiple range test. Analyses did not reveal significant effects of sex or significant interactions between fixed effects, therefore they are not reported in the tables. All calculations were made with Statistica 10.0 (StatSoft, 2011).

RESULTS AND DISCUSSION

No significant differences were found in the average live weight of rabbits in any of the groups. Also no significant differences were observed in daily body weight gains. No deaths were recorded throughout the experiment, and no visible symptoms of disease were observed (Table 3).

Similar final body weights of rabbits, indicate that all diets were well balanced, which points to high availability of nutrients, including amino acids contained in RSM, WLS and PS. Average daily gains were similar in all groups and had no significant effect on the final body weights of rabbits.

In our study and in experiments performed by other authors, RSM, PS and WLS used as substitutes for SMB had no adverse influence on the growth rate of rabbits. Gasmi-Boubaker et al. (2007), Strychalski et al. (2014) and Gugolek et al. (2015) demonstrated that dietary inclusion of RSM at 7% and 5% had no effect on productivity. In a study by Volek and Marounek (2009), dietary inclusion of 15% WLS did not affect the body weights or average daily gains of rabbits, in comparison with animals fed SBM-based diets. Moreover, Volek et al. (2014) used a relatively high inclusion level (25%) of whole WLS without any adverse effects on milk production, milk composition, feed intake, body weights of rabbit does or viability of their offspring. An improvement in digestive health was also noted in rabbits fed the WLS-based diet ad libitum, compared with those fed the SBM diet (Uhlirova et al., 2015).

According to Lounaouci-Ouyed, Berchiche and Gidenne (2014), pea seeds can be added to rabbit diets at up to 30% with no negative influence on average daily gain relative to the values reported in rabbits receiving SBM. The replacement of SBM with RSM, WLS and PS had no negative effect on the growth performance of Popielno White rabbits, either (Zwolinski et al., 2017). However, the performance parameters obtained in the above study were lower than those determined in our experiment, which resulted from differences between Popielno White rabbits and Hyplus rabbits.

Hematology and blood biochemistry parameters of rabbits are presented in Table 4. The highest LYM (%) value was observed in group C; the differences relative to groups E1 and E2 reached 20.5% and 7.4%, respectively, and the former was statistically significant. GRA (%) count was significantly higher in group E1 than in groups C and E2. Significant differences were also noted in PCT whose values were determined at 0.60% in group C, 0.14% in group E1 and 0.13% in group E2.

No significant differences were found between groups in blood biochemical parameters, except for GTP which was highest in group C. The levels of GTP and GOT were influenced by the SBM content of diets. A higher GTP level was noted in rabbits fed a diet with a lower SBM content, whereas a higher GOT level was observed in animals fed a diet with a higher SBM content. Blood cholesterol and total protein levels were highest in group E2 and lowest in group C. The highest and lowest FFA concentrations were noted in group E2 and group C, respectively. Triglyceride and glucose levels were higher in groups C and E2 than in group E1.

Considerable differences related to age, gender and diet were observed in the blood parameters of rabbits (Tumova et al., 2007; Etim et al., 2014). The values presented in Table 3, excluding cholesterol, are similar to those reported by Tumova et al. (2007; 2013). An absence

Table 3: Growth performance of rabbits (mean±SEM, n=30).

Specification	Group			P
	C	E1	E2	
Live weight 35 day (g)	954 ± 10	949 ± 11	955 ± 9	0.913
Live weight 56 day (g)	1930 ± 38	1999 ± 43	2047 ± 46	0.111
Live weight 84 day (g)	3141 ± 61	3133 ± 100	3186 ± 59	0.868
Daily weight gains 35-56 days (g)	46 ± 3	50 ± 3	52 ± 3	0.474
Daily weight gains 57-84 days (g)	42 ± 3	42 ± 3	41 ± 3	0.915
Daily weight gains 35-84 days (g)	44 ± 5	45 ± 4	46 ± 5	0.898
Feed intake 35-84 days (kg)	5.93 ± 0.21	5.88 ± 0.19	5.94 ± 0.19	0.865
Feed conversion ratio (kg/kg)	2.71 ± 0.10	2.69 ± 0.07	2.66 ± 0.04	0.793
Mortality (%)	0	0	0	-

No statistically significant differences.

of significant difference in the analyzed blood parameters indicates that a combination of RSM, WLS and PS as substitutes for SBM exerted no adverse effects on rabbits.

The content of antinutritional factors in experimental diets, which presumably originated from WLS (4 and 8%), was not determined, but it could not be high since it had no adverse influence on the growth performance or blood parameters of rabbits. According to Hedges and Lister (2006), legume seeds may also deliver beneficial effects. Saponins present in peas contribute to nutrient absorption and regulate gut microbiota.

The slaughter parameters in rabbit carcasses is shown in Table 5. No significant differences were observed in the values of the analyzed parameters between group C vs. groups E1 and E2.

According to Attia et al. (2015), feeding regimes generally have no significant effect on the weights of internal organs in rabbits, except for liver size, which is consistent with the findings of Tumova et al. (2007). In a study by Rubio et al. (1999), dietary supplementation with legume seeds had no influence on body weight or the weights of internal organs in rabbits, excluding liver weight. Animals fed a legume-based diet had lower relative liver weights than control animals. In contrast, in our experiment rabbits fed a diet with the highest content of legume seeds had heavier livers.

Comparable dressing percentage values point to similarities in muscle growth, fat deposition and gastrointestinal tract development in rabbits from all groups. Similar dressing percentage values, calculated by the same method, were reported by Gugolek et al. (2015). In typical broiler rabbits,

Table 4: Hematology and blood biochemistry parameters of rabbits (mean±SEM, n=10).

Specification	Group			P
	C	E1	E2	
WBC (10^9/l)	6.37 ± 0.76	5.90 ± 0.89	5.31 ± 0.69	0.638
LYM (%)	61.8 ± 5.4[a]	41.3 ± 5.3[b]	54.4 ± 7.0	0.018
MID (%)	7.64 ± 1.94	7.04 ± 1.99	6.68 ± 1.82	0.938
GRA (%)	30.6 ± 6.6[b]	51.7 ± 6.1[a]	39.0 ± 7.9[b]	0.008
RBC (10^{12}/l)	6.21 ± 0.22	6.22 ± 0.21	6.40 ± 0.15	0.756
HGB (mmol/l)	11.0 ± 0.7	12.1 ± 0.5	12.2 ± 0.2	0.154
HCT (l/l)	0.39 ± 0.18	0.39 ± 0.15	0.39 ± 0.09	0.970
MCV (fl)	61.3 ± 0.6	62.0 ± 1.4	61.0 ± 0.7	0.738
MCH (pg)	17.8 ± 1.1	19.4 ± 0.4	19.1 ± 0.3	0.266
MCHC (d/dl)	29.2 ± 1.9	31.3 ± 0.2	31.3 ± 0.2	0.299
RDWc (%)	21.5 ± 0.7	20.8 ± 0.7	20.2 ± 0.6	0.337
PLT (10^9/l)	186 ± 54	199 ± 57	197 ± 87	0.989
PCT (%)	0.60 ± 0.47[a]	0.14 ± 0.04[b]	0.13 ± 0.06[b]	0.001
MPV (fl)	7.68 ± 0.18	7.50 ± 0.17	7.39 ± 0.15	0.485
PDWc (%)	34.6 ± 0.7	33.2 ± 0.6	33.1 ± 0.5	0.190
TP (g/l)	52.4 ± 3.6	57.9 ± 1.4	59.8 ± 0.2	0.068
Glu (mmol/l)	7.12 ± 0.20	6.60 ± 0.17	7.03 ± 0.18	0.122
Chol (mmol/l)	1.23 ± 0.04	1.25 ± 0.04	1.38 ± 0.10	0.217
TG (mmol/l)	0.99 ± 0.10	0.72 ± 0.04	0.99 ± 0.12	0.087
FFA (mmol/l)	0.58 ± 0.03	0.56 ± 0.04	0.49 ± 0.03	0.134
GTP (U/l)	37.5 ± 3.2[b]	41.5 ± 5.1[b]	54.7 ± 3.2[a]	0.012
GOT (U/l)	48.7 ± 5.9	43.5 ± 1.8	42.5 ± 1.6	0.448
GGTP (U/l)	12.0 ± 0.6	10.7 ± 0.3	10.7 ± 0.4	0.065

[a,b]Mean values within rows with no common superscript are different at p≤0.05.

dressing percentage without the head and giblets generally exceeds 50% (Zita et al., 2007; Volek; Marounek, 2009; Tumova et al., 2013; Strychalski et al., 2014).

The contribution of cuts in rabbit carcasses was similar to that reported by Daszkiewicz et al. (2012) and Gugolek et al. (2015). In our study, carcass quality parameters were comparable in all groups and typical of broiler rabbits under intensive production conditions. Also in a study by Zwolinski et al. (2017), diets similar to those used in our experiment did not induce significant changes in the slaughter yield of Popielno White rabbits.

The complete replacement of SBM with RSM, WLS and PS (E2) significantly increased the activity of bacterial β-glucosidase, α-arabinopyranosidase, α-arabinofuranosidase and β-xylosidase, relative to group C in the caecum (Table 6). Rabbits assigned to dietary

Table 5: Slaughter parameters of rabbits (mean±SEM, n=30).

Specification	Group			P
	C	E1	E2	
Pre-slaughter weight (g)	3141 ± 61	3133 ± 100	3186 ± 59	0.868
Hot carcase weight with head (g)	1904 ± 39	1881 ± 69	1911 ± 37	0.906
Hot carcase weight without head (g)	1741 ± 38	1722 ± 65	1753 ± 35	0.901
Head (g)	159 ± 3	156 ± 4	154 ± 3	0.647
Liver (g)	75 ± 3	75 ± 3	78 ± 2	0.797
Kidneys (g)	19 ± 1	20 ± 1	20 ± 1	0.860
Heart (g)	9 ± 1	11 ± 1	10 ± 1	0.466
Lungs (g)	16 ± 1	16 ± 1	15 ± 1	0.686
Skin and legs (g)	506 ± 18	534 ± 22	516 ± 14	0.558
Cold carcase weight without head (g)	1662 ± 35	1648 ± 62	1673 ± 34	0.929
Inedible unit weight (g)	472 ± 10	482 ± 12	495 ± 14	0.313
Dressing percentage (%)	55.4 ± 0.4	54.8 ± 0.6	55.0 ± 0.4	0.599
Forepart (g)	637 ± 15	628 ± 26	622 ± 16	0.869
Loin (g)	423 ± 10	421 ± 18	436 ± 10	0.688
Hind part (g)	601 ± 11	600 ± 19	615 ± 10	0.710

No statistically significant differences.

Table 6: Bacterial enzyme activity in the caecal digesta of rabbits fed experimental diets* (mean±SEM; n = 8).

Specification	C	E1	E2	P
α-glucosidase	5.02 ± 1.25	3.51 ± 0.43	3.36 ± 0.45	0.181
β-glucosidase	2.94 ± 0.60^{b}	6.08 ± 1.23^{ab}	8.77 ± 1.67^{a}	0.005
α-galactosidase	15.5 ± 1.7^{a}	12.3 ± 1.3^{ab}	10.9 ± 0.9^{b}	0.029
β-galactosidase	24.7 ± 3.1^{a}	19.1 ± 1.5^{ab}	13.5 ± 1.4^{b}	0.002
β-glucuronidase	104 ± 8^{a}	92.1 ± 5.9^{a}	71.2 ± 3.6^{b}	0.001
α-arabinopyranosidase	1.16 ± 0.10^{b}	1.46 ± 0.09^{ab}	1.56 ± 0.11^{a}	0.048
α-arabinofuranosidase	1.21 ± 0.17^{b}	1.81 ± 0.15^{ab}	2.04 ± 0.27^{a}	0.012
β-xylosidase	1.51 ± 0.15^{b}	1.89 ± 0.07^{ab}	2.02 ± 0.17^{a}	0.044
β-cellobiosidase	1.28 ± 0.11^{b}	1.73 ± 0.14^{a}	1.78 ± 0.14^{a}	0.042

*μmol/h/g caecal digesta

^{a,b} Mean values within rows with no common superscript are different at p≤0.05.

treatments E1 and E2 were characterized by significantly higher activity of bacterial β-cellobiosidase in the caecal digesta. The complete replacement of SBM with other sources of dietary protein (group E2) caused a significant decrease in the activity of α- and β-galactosidases in the caecum as compared with group C. The activity of bacterial β-glucuronidase in the caecum decreased in dietary treatment E2. Both complete and partial substitution of SBM led to a significant increase in caecal total VFA and acetic acid pools (Table 7). Dietary treatment E2 was also associated with a significant increase - compared to C group - in the caecal iso-valeric acid pool.

The changes in the enzymatic activity of gut microbiota, observed in our study, could be due to various reasons, including different amounts of undigested starch, non-starch polysaccharides and other dietary ingredients reaching the final segment of the digestive tract in rabbits. An analysis of bacterial enzymatic activity in the caecal digesta in group E1 and E2 revealed increased mobilization of microbiota aimed at producing enzymes which may enable them to obtain additional energy from less digestible fiber fractions through fermentation. Higher activities of selected enzymes, including α-arabinopyranosidase, α-arabinofuranosidase, β-xylosidase and β-cellobiosidase, were observed. For instance, specific enzymes are required to degrade NSPs present both in cereals and protein sources used in this study, namely arabinans containing terminal arabinofuranoses as well as internal arabinopyranoses. Degradation processes in the large intestine would be limited without those and others enzymes. Of course, some changes in the bacterial enzymatic activity could be ascribed to different content of cereals in the feed mixtures, as, for instance, wheat and barley contain less cellulose and more xylans in comparison to SBM, pea and lupine seeds (Bach Knudsen, 2014). Partial and complete replacement of SBM with other protein sources (RSM, WLS and PS), and associated changes in dietary cereals proportion, led to a gradual increase in dietary CF and ADF levels (least digestible fiber fractions). This could be considered as an adaptation mechanism of rabbit's microbiota to obtain additional energy from the large intestine via gut fermentation. The higher activity of β-glucosidase in group E2 could be related to an increased content of dietary polyphenolic compounds provided by RSM, WLS and PS. β-Glucosidase is involved into deglycosylation processes, which is a critical step in the absorption of dietary polyphenols (Juskiewicz et al., 2011). The experimental substitution of dietary SBM decreased caecal β-glucuronidase activity, but increased the activity of other bacterial enzymes, which suggests that dietary treatment E2 exerted selective beneficial effects on cecal microbiota. The activity of β-glucuronidase is often used as a marker of pathogenic microbiota with undesirable effects on metabolic processes (Klewicka; Zdunczyk; Juskiewicz, 2009).

The effects of enhanced bacterial caecal VFA production following the administration of SBM substitutes were primarily associated with an increase in the acetic acid pool. It should be noted that the sum of putrefactive VFAs produced in the caecum did not differ between groups. Those acids are considered as useful indicators describing the rate of anaerobic bacterial polypeptide and amino acid fermentation in the lower gastrointestinal tract (Gugolek et al., 2015). Taking into account both changes in caecal enzymatic activity and VFA

Table 7: Volatile fatty acids produced (VFA pool) by microbiota in the caecum of rabbits fed experimental diets (mean±SEM; n = 8).

	C	E1	E2	P
VFA, μmol/kg of BW				
acetic	990 ± 58^{b}	1233 ± 108^{a}	1334 ± 104^{a}	0.044
propionic	115 ± 12	118 ± 6.6	124 ± 14	0.612
iso-butyric	9.96 ± 2.54	8.22 ± 1.03	10.2 ± 0.7	0.426
butyric	54.7 ± 7.6	52.6 ± 6.8	55.7 ± 5.4	0.758
iso-valeric	2.73 ± 0.64^{b}	4.23 ± 0.64^{ab}	5.06 ± 0.92^{a}	0.048
valeric	10.1 ± 1.4	10.3 ± 0.8	10.0 ± 1.2	0.858
total putrefactive VFA	22.8 ± 4.1	22.8 ± 1.7	25.3 ± 2.0	0.538
total VFA	1182 ± 88^{b}	1427 ± 143^{a}	1538 ± 138^{a}	0.049

^{a,b} Mean values within rows with no common superscript are different at p≤0.05
BW- body weight; putrefactive VFA = the sum of iso-butyric, iso-valeric and valeric acids;
VFA – volatile fatty acid.

production observed in the present study, the experimental application of RSM, WLS and PS should be considered as beneficial to the host's intestinal environment

CONCLUSIONS

The results of this study indicate that dietary SBM can be replaced (up to 15% of the diet) with a mixture of RSM, WLS and PS without any negative effect on growth rate, selected morphological and biochemical blood parameters of Hyplus rabbits. The comparable production parameters among control and experimental rabbits fed diets containing SBM or its substitutes could be partially ascribed to beneficial changes in the enzymatic activity of caecal microbiota, which have a positive influence on fermentation processes in the lower gastrointestinal tract.

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