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Effectiveness of emulsifier and carbohydrase in *Lupinus albus* seed-rich diets on digestibility, some physiological indicators and performance of growing pigs

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ABSTRACT. The study aimed to evaluate the impact of emulsifier and/or carbohydrase additives on the production performance of growing pigs offered a diet with a high proportion of white lupine seeds cv. Butan. The experiment involved 49 Naïma × P76 male piglets with an initial body weight of 10.5 kg divided into seven groups. The control group was fed a diet without any additives, whereas the remaining groups received diets with the addition of emulsifier, β glucanase, and β-xylanase separately or in combination. The apparent ileal digestibility coefficients of crude protein were significantly improved ($P < 0.05$) by all additives, although no significant differences were observed in dry matter and ether extract digestibility ($P > 0.05$). Both to the performance results and ammonia concentration, pH, and digesta viscosity ($P > 0.05$), were not different when these feed additives were used. The most promising effects on nutrients digestibility and performance were obtained were emulsifier, β-glucanase and their blend were added to the feed mixtures.

Introduction

Lupine seeds contain many valuable nutrients that can be utilised in animal feeds and partially or completely replace other commonly use proteins, such as soybean meal (SBM). White lupine (*Lupinus albus* L.) seeds have a protein content ranging from 33 to 47%, depending on variety and location, and contain a higher fat content (~10%) compared to other lupine species (Kasprowicz-Potocka et al., 2017). However, they also harbour high levels of anti-nutritional factors (ANF), which can negatively affect production outcomes. The most common ANF identified in white lupin seeds include non-starch polysaccharides (NSP), raffinose family oligosaccharides (RFO), and alkaloids (Kasprowicz-Potocka et al., 2016). However, according to some studies, incorporating white lupine seeds into piglet diets may adversely affect animal rearing parameters (Mieczkowska et al., 2004; Jezierny et al., 2010; Kasprowicz-Potocka et al., 2016). White lupine seeds contain approx. 14–15% crude fibre and about 10% RFO (Kasprowicz-Potocka et al., 2022). Pigs metabolise NSP and RFO poorly due to the lack of specific endogenous enzymes for their decomposition. Incorporating exogenous enzymes, such as carbohydrases, into animal diets can effectively degrade carbohydrates into simple sugars, and thus reduce digesta viscosity, and enhance nutrient availability (Lee et al., 2018; Duarte et al., 2019; Aranda-Aguirre et al., 2021).

The results by Yin et al. (2001) demonstrated that the addition of enzymes such as β-glucanase, xylanase, and protease improved the apparent ileal digestibility (AID) of dry matter (DM), gross energy, and the content of crude protein (CP), amino acids (AA), neutral detergent fibre (NDF) and total NSP. A study performed on piglets showed that xylanase in the diet of weaned pigs increased the average daily gains (ADG) (Jezierny et al., 2010). However, Passos et al. (2015) reported positive effects of xylanase supplementation on AID, but not on feed efficiency.

The primary cost component in feed formulation is energy. However, young animals have limitations in the absorption of certain nutrients, such as fats, due to low levels of native lipase and limited bile salt production in the early developmental stages (Gu and Li, 2003). Moreover, soluble NSP are also known to hinder fat digestion by impeding the diffusion of digestive enzymes and bile acids (Wealleans et al., 2021). The efficiency of fat digestion directly impacts the energy animals derive from this dietary component. While white lupine seeds appear to be a promising fat source, studies frequently indicate poor digestion of ether extract from these seeds (Mieczkowska et al., 2004; Pisarikova et al., 2008; Jezierny et al., 2010; Kasprowicz-Potocka et al., 2017). The addition of an emulsifier may reduce the surface tension between immiscible aqueous and fat phases, thereby enhancing lipid digestion and absorption. Supplementation with exogeneous emulsifier can also increase the absorption of other nutrients, including proteins, thus improving poultry and pig performance (Zhao et al., 2015; Bai et al., 2019; Kubiś et al., 2020; Wiśniewska et al., 2023). The effectiveness of emulsifiers is estimated to be 20200 times greater than the emulsifying properties of animal bile.

While the content of antinutritional substances in white lupine seeds generally tends to be lower than in other lupine species, a diet containing more than 150 g/kg of *L. albus* seeds has been shown to reduce pig gain and feed intake compared to other lupines (Jezierny et al., 2010; Kasprowicz-Potocka et al., 2016; 2017). Despite this, current data concerning the use of white lupine in pig nutrition are still unclear, and the reason for lower porcine tolerance is yet unknown (Kasprowicz-Potocka et al., 2017). Therefore, we hypothesised that employing external enzymes to degrade structural carbohydrates could partially reduce the negative effects of white lupine seeds. Moreover, the inclusion of an emulsifier may release fat and other nutrients, thereby improving the overall digestibility of the seeds. The objective of this study was to assess the effectiveness of supplementing a diet high in white lupine seeds with carbohydrases and/or emulsifiers on production outcomes, nutrient digestibility, and selected physiological parameters of the digestive tract in growing pigs.

Material and methods

White lupine seeds

White lupine seeds (*Lupinus albus* L.) var. Butan were obtained for the experiment from the Plant Breeding Station in Przebędowo (IHAR, Poland).

Ethical Statement

All experimental procedures conducted in this study adhered to the guidelines of Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes (EU Directive 2010/63/EU, 2010). The Local Ethics Committee in Poznań approved the experiment under Resolution No. 43/2011 of May 15, 2011.

Animals, diets, and experimental design

The experiment involved 49 Naïma × P76 male piglets (AGRO-PIG, Kamionna, Poland) with an initial body weight (IBW) averaging 10.5 kg. The pigs were randomly assigned to seven dietary treatments (Table 1), with each treatment group consisting of seven individuals (n = 7). The groups of animals were housed in separate boxes on straw for 28 days, with each group representing 1 replicate. The experiment was divided into two equal periods, the first spanning from days 1 to 14, and the second from days 15 to 28.

Piglets were provided with complete feed mixtures as a mash form and had unrestricted access to water. Pigs were offered feed mixtures *ad libitum*, and feed intake was monitored. The experimental design is shown in Table 1. Glyceryl polyethylene glycol ricinolate/PEG-35 castor oil (CAS: 61791-12-6) was added at a rate of 0.04% as an emulsifier (Syskem Chemie GmbH, Wuppertal, Germany). Additionally, two enzymes were used: enzyme 1 – heat-stable endo-1,3(4)-β-glucanase (EC 3.2.1.6) from *Aspergillus aculeatus* (CBS 589.94) at a rate of 0.04%, and enzyme 2 – endo-1,4-β-xylanase, endo-1,3(4)-β-glucanase, endo-1,4-β-glucanase (EC 3.2.1.8; EC 3.2.1.6; EC 3.2.1.4) from *Trichoderma reesei* (ATCC 74444) at an 0.03% inclusion rate (DSM, Mszczonów, Poland). All the diets were formulated according to the Dietary recommendations and the nutritional value of feed for pigs (Grela and Skomiał, 2015). Titanium dioxide (TiO_2) at a concentration of

Table 1. Composition and nutrient contents of the experimental diets

Component, % / Group	1	2	3	4	5	6	7
Wheat	49.50	49.46	49.46	49.42	49.47	49.43	49.43
Soybean meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00
White lupine seeds	16.00	16.00	16.00	16.00	16.00	16.00	16.00
Maize	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Phosphate 1-Ca	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Limestone	1.00	1.00	1.00	1.00	1.00	1.00	1.00
NaCl	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Soybean oil	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Premix*	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine HCl	0.41	0.41	0.41	0.41	0.41	0.41	0.41
DL-Threonine	0.17	0.17	0.17	0.17	0.17	0.17	0.17
DL-Tryptophan	0.06	0.06	0.06	0.06	0.06	0.06	0.06
TiO_2	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Emulsifier	–	0.04	–	0.04	–	0.04	–
Enzyme 1	–	–	0.04	0.04	–	–	0.04
Enzyme 2	–	–	–	–	0.03	0.03	0.03
Calculated nutritional value							
Metabolizable energy, MJ/kg				13.8			
Digestible lysine, g/kg				10.5			
Digestible methionine, g/kg				3.49			
Digestible threonine, g/kg				6.75			
Digestible tryptophan, g/kg				2.07			
Crude protein, g/kg				168.0			
Ca, g/kg				7.79			
P, g/kg				6.16			
Digestible P, g/kg				3.69			
Na, g/kg				1.36			

* contained the following component quantities per kg: mg: Fe 75, Cu 20, Co 0.3, Mn 30, Zn 75, I 0.6, Se 0.15, vit. E 52.5, vit. K_3 1.1, vit. B_1 1.1, vit. B_2 3.0, vit. B_6 2.25, choline chloride 200, pantothenic acid 7.5, nicotinic acid 15, folic acid 1.5; IU: vit. A 7 500, vit. D_3 1500; µg: vit. B_{12} 18.5, biotin 75; g: Ca 1.3; antioxidants (butylated hydroxyanisole, butylated hydroxytoluene); emulsifier – glyceryl polyethylene glycol ricinolate/ PEG-35 castor oil (CAS: 61791-12-6), enzyme 1 – endo-1,3(4)-β-glucanase, enzyme 2 – endo-1,4-β-xylanase, endo-1,3(4)-β-glucanase, endo-1,4-β-glucanase

3 g/kg (Police, Poland) was included in all diets as an indigestible marker.

During the experimental period, the health status of the pigs, including their overall well-being and faecal consistency, was monitored twice daily. The body mass of all pigs was recorded on days 1, 14, and 28 to calculate body weight gain (BWG). Additionally, the feed intake (FI) of each group was monitored on days 14 and 28 of the experiment, followed by the calculation of the feed conversion ratio (FCR). Upon conclusion of the experiment, the pigs were humanely stunned using an electric shock. Directly after euthanasia, intestinal contents (ileum and caecum) were collected from all animals in each group (n = 7) for analysis. Digesta were collected separately from each individuum and frozen at −80 °C.

Chemical analysis

The representative samples of white lupine seeds were ground and passed through a sieve with a mesh size of 0.1 or 0.05 mm. Digesta samples were frozen at −80 °C, freeze-dried, and ground for analysis. Both seeds and digesta samples were analysed in duplicate for the content of DM using the gravimetric method, crude protein (CP) using the Kjeldahl method, while ether extract (EE), crude fibre (CF), crude ash (CA), acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium, and phosphorus contents were assayed using methods 934.01, 976.05, 920.39, 978.10, 942.05, 973.18, 2002.04, 984.27 and 965.17, respectively, according to AOAC (2007).

Nitrogen-free extractives (NFE) were calculated using the following equitation:

$$NFE\ (\%) = DM\ (\%) - (CP\ (\%) + CA\ (\%) + CF\ (\%) + EE\ (\%)).$$

Protein digestibility was analysed using the method described by Hsu et al. (1977), employing a multienzyme *in vitro* system consisting of trypsin, chymotrypsin, and peptidase. Phytate phosphorus

was determined calorimetrically using the Haug and Lantzsch (1983) method with modifications. A spectrophotometer (Marcel Media, Poland) was utilised to measure absorbance after complexing phytate-P with iron salt and piperidine. Metabolizable energy was calculated according to the methodology outlined in the 'Dietary Recommendations and the Nutritional Value of Feed for Pigs' (Grela and Skomiał, 2015). The concentration of amino acids (AA) was measured using an AAA-400 Automatic Amino Acid Analyser (Ingos Ltd., Prague, Czech Republic), with ninhydrin applied for post-column derivatisation. The samples were hydrolysed with 6 M HCl for 24 h at 110 °C following procedure 994.12 of AOAC (2007). The TiO_2 content was determined according to the method described by Short et al. (1996), with samples prepared according to the procedure described by Myers et al. (2004). For TiO_2 content determination, the samples were digested in concentrated H_2SO_4 for 2 h, followed by the addition of 30% H_2O_2, and absorbance measurements at 410 nm using a spectrophotometer (Marcel Media, Poland). RFO were extracted and analysed by HPLC as described by Lahuta et al. (2018). Digesta samples were collected approx. 50 cm before the ileocecal valve, mixed, and their pH was measured using a 301 pH-meter (Hanna Instruments, Vila do Conde, Portugal). Digesta viscosity was measured by centrifuging the samples at 10 000 × g for 10 min at 4 °C. The supernatant was collected and viscosity was measured using a Brookfield Digital DV-II + cone/plate viscometer (Brookfield Engineering Laboratories, Stoughton, MA, USA) maintained at 37 °C with a shear rate of 60 s. Ammonia content in the ileum and caecum were analysed using the spectrometric method employing Nessler's reagent (POCh, Gliwice, Poland) (Szumacher-Strabel et al., 2022).

Digestibility calculation

The apparent ileal digestibility (AID) coefficients of nutrients in diets were calculated using the following formula:

$$\text{AID (\%)} = 100 - 100 \left(\frac{\text{\% of marker in feed}}{\text{\% of marker in faeces}} \times \frac{\text{\% of nutrient in feaces}}{\text{\% of nutrient in feed}} \right).$$

Statistical analysis

The results were analysed using ANOVA to assess the impact of the experimental diets on the parameters studied. Differences between means were compared using Duncan's test at $P < 0.05$ implemented in SAS 9.3 statistical software, (SAS Institute Inc., Cary, NC, USA). Values presented in the tables are presented as means, accompanied by standard error (SEM) and standard deviation (SD).

Results

Chemical composition of white lupine seeds

Elemental chemical composition, selected ANF contents, and AA profile of white lupine seeds are presented in Table 2.

Seeds of the white lupine var. Butan used in the experiment were characterised by a high DM content, with approx. 31% CP, of which 75.6% was determined as digestible protein. The seeds also contained almost 10% EE and about 13% CF. The calculated ME value for pigs was approx. 13.8 MJ/kg. Methionine content in the seeds was relatively low, at about 0.9 g/100 g

Table 2. Chemical composition of white lupine seeds (n = 4)

Nutrients	%
Dry matter	92.31
Crude ash	3.71
Crude protein (CP)	31.11
Digestible CP (*in vitro*)	23.52
Ether extract	9.92
Nitrogen-free extractives	34.38
Crude fibre	13.19
Neutral detergent fibre	22.47
Acid detergent fibre	19.68
Ca	0.26
P	0.46
Metabolizable energy*, MJ/kg	13.79
Amino acid profile	g/100 g of proteins
aspartic acid	10.76
threonine	3.77
serine	5.18
glutamic acid	19.81
proline	4.21
cystine	1.25
glycine	4.16
alanine	3.66
valine	4.22
methionine	0.89
isoleucine	4.36
leucine	7.21
tyrosine	4.08
phenylalanine	3.92
histidine	2.68
lysine	6.01
arginine	11.08
Antinutrients	%
total raffinose family oligosaccharides (RFO)	9.56
raffinose, %RFO*	3.56
stachyose, %RFO*	84.21
verbascose, %RFO*	12.24
phytate phosphorus (P Phy)	0.22
P Phy/Total P	47.82
viscosity	1.03 cP

* calculated value

protein, while lysine content exceeded 6.0 g/100 g protein, and threonine approached 3.8 g/100 g. Seed analysis for the presence of RFO revealed a total content of these sugars at approx. 10%, with stachyose constituting the highest proportion, followed by verbascose and raffinose. The content of phytate P was 0.22% DM, representing nearly 48% of total P. Seed viscosity reached just over 1 cP.

Animal experiment

Although no significant differences ($P > 0.05$) were observed in performance results among the groups, significant numerical differences were observed (Table 3). Throughout the experimental period, the most favourable performance results were obtained in group 2, fed a feed mixture with an emulsifier. Conversely, groups 5–7 demonstrated the lowest BWG, and unfavourable FCR.

Significant differences ($P < 0.05$) were noted in the AID coefficients of CP, whereas AID for DM and EE did not differ significantly between the groups (Table 4). The AID of dietary CP was significantly higher in groups 2–6 compared to control 1, but the highest value was observed in group 4. Generally, the highest values of the AID coefficient for dietary EE (and DM) were recorded in groups 2 and 3.

No significant differences ($P > 0.05$) were observed in the physiological parameters of the ileal and caecal digesta for all evaluated variables (Table 5). The lowest pH value was measured in group 4, fed a diet with emulsifier and enzyme 2 supplementation. The lowest viscosity of the ileal digesta was recorded in pigs from group 3, fed a diet with enzyme 1 addition, while the highest value was noted in groups 4 and 7. Additionally, the highest pH value of the ileal digesta was determined for pigs from group 7. The lowest ammonia content in

Table 3. Body weight gain, feed intake, and feed efficiency of pigs (n=7)

Group	1	2	3	4	5	6	7	SD	SEM	*P*-value
Emulsifier	–	+	–	+	–	+	-			
Enzyme 1	–	–	+	+	–	–	+			
Enzyme 2	–	–	–	–	+	+	+			
Initial body mass (BM)	10.63	10.62	10.61	10.56	10.49	10.52	10.61	0.59	0.211	0.928
Final BM	22.51	23.95	22.95	22.88	21.63	22.26	21.92	1.48	0.398	0.488
Body weight gain, kg										
days 1–14	4.75	5.95	4.78	4.79	4.55	4.78	4.35	1.16	0.160	0.215
days 15–28	7.13	7.36	7.55	7.53	6.59	6.97	6.96	1.17	0.358	0.711
days 1–28	11.88	13.32	12.33	12.32	11.14	11.74	11.31	2.06	0.288	0.513
Feed intake, kg										
days 1–14	9.31	10.42	9.75	8.93	9.52	9.10	9.72	1.60	0.220	0.674
days 15–28	14.20	15.15	14.84	14.45	15.20	14.49	15.41	1.85	0.259	0.870
days 1–28	23.51	25.56	24.59	23.38	24.73	23.59	25.13	3.25	0.455	0.821
Feed conversion ratio, kg/kg										
days 1–14	2.02	1.80	2.08	1.90	2.16	1.93	2.33	0.42	0.059	0.289
days 15–28	2.00	2.09	2.03	1.94	2.32	2.10	2.27	0.33	0.046	0.249
days 1–28	2.00	1.95	2.04	1.91	2.24	2.03	2.27	0.32	0.045	0.231

results in the table are expressed as mean values; enzyme 1 – endo-1,3(4)-β-glucanase, enzyme 2 – endo-1,4-β-xylanase, endo-1,3(4)-β-glucanase, endo-1,4-β-glucanase; SD – standard deviation, SEM – standard error of the mean; significance at $P \leq 0.05$

Table 4. Apparent ileal digestibility coefficients (%) of dry matter (DM), crude protein (CP), and ether extract (EE) of the diets (n = 7)

Variable/Group	1	2	3	4	5	6	7	SD	SEM	*P*-value
Emulsifier	-	+	–	+	–	+	–			
Enzyme 1	–	–	+	+	–	–	+			
Enzyme 2	–	–	–	–	+	+	+			
DM, %	93.80	95.64	95.54	94.28	95.05	94.67	95.22	1.93	0.28	0.500
CP, %	47.69^{b}	62.18^{a}	58.80^{a}	63.28^{a}	62.27^{a}	62.26^{a}	57.38^{ab}	9.45	1.48	0.026
EE, %	62.98	68.79	68.87	66.75	62.98	61.55	66.47	9.99	1.51	0.806

results are reported as mean values; enzyme 1 – endo-1,3(4)-β-glucanase, enzyme 2 – endo-1,4-β-xylanase, endo-1,3(4)-β-glucanase, endo-1,4-β-glucanase; SD – standard deviation, SEM – standard error of the mean; ^{ab} means with different superscripts within a row are significantly different at $P \leq 0.05$

Table 5. Digesta pH, viscosity and ammonia concentration in the ileum and caecum, (n = 7)

Variable/Group	1	2	3	4	5	6	7	SD	SEM	*P*-value
Emulsifier	–	+	–	+	–	+	–			
Enzyme 1	–	–	+	+	–	–	+			
Enzyme 2	–	–	–	–	+	+	+			
pH										
ileum	5.83	5.69	5.44	5.29	5.69	5.88	6.16	0.60	0.08	0.122
Viscosity (cP)										
ileum	3.22	2.89	2.26	3.29	2.48	2.64	3.36	0.96	0.14	0.201
Ammonia (mmol/l digesta)										
ileum	8.39	9.17	11.40	11.33	10.65	11.29	11.40	3.01	0.43	0.318
caecum	9.65	10.22	10.23	7.61	11.85	7.61	10.43	4.31	0.62	0.509

results are reported as mean values; enzyme 1 – endo-1,3(4)-β-glucanase, enzyme 2 – endo-1,4-β-xylanase, endo-1,3(4)-β-glucanase, endo-1,4-β-glucanase; SD – standard deviation, SEM – standard error of the mean; significance at $P \leq 0.05$

the ileal digesta was observed in the control group, whereas in the caecum for groups 4 and 6. The highest ammonia content in the ileum digesta was recorded in pigs from groups 3 and 7, while in the caecum in animals from group 5.

Discussion

According to previous studies, lupin seeds exhibit significant variations in chemical composition and, consequently, in nutritional value depending on the plant origin background (Kasprowicz-Potocka et al., 2017; Mierlita et al, 2018). White lupin seeds used in the present experiment contained approx. 31% CP, and a similar result was described in the 'Swine Nutrition Requirements' (Grela and Skomiał, 2015). Conversely, other authors determined CP content at 35–40% in different white lupine seeds (Calabrò et al, 2015; Mierlita et al., 2018). Abreu and Bruno-Soares (1998) observed that the high CP content was partially caused by a low, often marginal, level of starch in the seeds, which may also be replaced by EE as the primary energy source. In our research, the EE content amounted to approx. 10%, similar to that obtained by Mierlita et al. (2018). Many authors have argued that the proportion of fat in the seeds depends on the variety and the harvest year (Calabrò et al, 2015). In our work, the CF content was over 13% and was consistent with the results of other authors, as was the amino acid profile, CA and ME (van Barneveld, 1999; Calabrò et al., 2015; Mierlita et al., 2018).

Particular attention in previous studies has been given to antinutritional substances present in white lupine seeds. The most commonly discussed are alkaloids, as they are naturally produced poisons by plants as part of their defensive mechanisms against aggressors. Alkaloid levels in DM of previously tested seeds was approximately 0.02% (Kasprowicz-Potocka et al., 2017). Currently, lupines cultivated worldwide are almost entirely sweet, accumulating <0.05% alkaloids in the seeds. The RFO content in the seeds of the var. Butan was relatively high, at about 10% DM, and was similar to those reported by Kasprowicz-Potocka et al. (2017, 2022). Phytates affect phosphorus bioavailability as they are permanently bound to the molecules and limit the utilisation of other elements forming chelate compounds. The content of phytic phosphorus in the seeds of the examined lupine was low, with phytate P levels not exceeding 50% in relation to total P. In contrast, DM phytate content in other white lupine varieties ranged from 0.25 to 0.6% (van Berneveld, 1999; Kasprowicz-Potocka et al., 2017). The level of alkaloids and phytate in white lupine analysed in the current study was very low and probably did not affect feed mixture quality.

The results of our previous studies concerning digestibility (Kasprowicz-Potocka et al., 2017) demonstrated that DM and CP of white lupine seeds were well digestible (82.8% for both), however, ether extract was poorly digestible (65.2%). In the present work, we also analysed the digestibility of diets and obtained similar results. The AID of ether extract was approx. 63% in the control diet, which was a very low value. Additionally, van Barneveld (1999) found that the digestibility coefficients of protein and fat were 85% and 67%, respectively. This might be attributed to the high digesta viscosity (3.22 cP) observed in the diet containing 16% white lupine seeds. A similar phenomenon was also observed in a previous experiment (Kasprowicz-Potocka et al. 2017), where digesta viscosity increased significantly with raising proportion of white lupine seeds in the diet. We found that when the diet contained 15% white lupine seeds, the viscosity was 2.18 cP, whereas when it was 32%, the viscosity increased to 4.50 cP. High viscosity may impede the digestion of nutrients

by specific enzymes by creating a physical barrier. However, the addition of emulsifier and/or carbohydrases did not clearly improve the digestibility of nutrients. All the additives applied in the current study, whether used separately or in combinations, improved the AID coefficient of DM by 0.87–1.84% ($P > 0.05$) and EE by 3.495.89 % ($P > 0.05$). On the other hand, all additives significantly enhanced the AID of CP by 9.6915.59%. This improvement is difficult to explain as it was not directly associated with reduced ileal digesta viscosity, which only occurred in groups where the additives were administered separately (groups 2, 3, and 5) and additionally in group 6 (emulsifier + mixture of xylanase and β-glucanases) ($P > 0.05$). A decrease in the viscosity of digestive contents following the addition of xylanase to the mixture was also documented by Bartelt et al. (2002). This effect can be explained by the fact that xylanase breaks down xylans and arabinans, which contribute to increased viscosity of digestive contents and reduced nutrient absorption.

The mode of action of xylanase and β-glucanase on CP digestibility may involve the degradation of polysaccharide cell walls, facilitating access of endogenous digestive enzymes to related components (Owusu-Asiedu et al., 2010; Moita and Kim 2022). This process also entails the breakdown of anti-nutritional factors present in plant material and the cleavage of chemical bonds resistant to animal enzymes. In contrast, supplementation with both enzymes (group 7) did not improve digestibility compared to the remaining additives, posing a challenge for interpretation. In our study, neither the emulsifier nor enzyme supplementation affect the pH, ammonia content in the digesta, and the viscosity of ileum digesta ($P > 0.05$).

Although the emulsifier and enzyme supplementation improved the CP digestibility ($P < 0.05$) in the present study, it did not improve the performance parameters ($P > 0.05$) of the pigs. Owusu et al. (2010) observed a dose-dependent effect of supplementation with a mixture of xylanase and β-glucanase on nutrient digestibility. Increasing the enzyme content from 50 g/kg to 200 g/kg in the pigs' diet resulted in improved nutrient digestibility ($P > 0.05$). Wiśniewska et al. (2023), using an emulsifier and enzymes in diets containing rapeseed meal for poultry, demonstrated lower total average feed intake and feed conversion ratio, along with higher nutrient digestibility. In another study, the combined supplementation of xylanase and emulsifier to the wheat and soybean-based poultry diets resulted in a more than threefold increase in NDF degradation, as well as improved digestibility of fatty acids and crude protein, ultimately resulting in more optimal BWG and FCR values.

The present study applied enzymes and the emulsifier at doses suggested by the manufacturers. However, it is likely that this type of diet containing a significant proportion of lupine seeds may require a higher concentration of additives. Moreover, the enzyme additive could be more precisely selected considering the low glucan content in white lupine seeds. Nevertheless, it should be emphasised that in the current study, some promising yet statistically insignificant effects were observed, such as improved growth and reduced FCR of animals from the group supplemented with emulsifier (group 2), β-glucanase (group 3), or both (group 4). Specifically, pigs administered emulsifiers had approx. 1.5 kg higher final body weight and 2.5% lower FCR, which could be important for producers.

Conclusions

In summary, white lupine seeds contain a high proportion of protein, ether extract, and nondigestible carbohydrates, which can exert antinutritive effects. Moreover, ether extract is poorly utilised in diets containing this component. The addition of the emulsifier and enzymes such as xylanase and/or β-glucanase and their mixtures had no significant effect on the performance parameters of growing pigs, despite significantly improving the apparent ileal digestibility of crude protein. The most promising effects were obtained for emulsifier and β-glucanase, as well as their combination, but further research in this area is needed.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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