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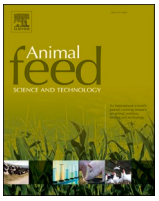

Potential of solid-state fermentation to enhance the nutritional value of oilseed cakes for poultry

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ABSTRACT

The objective of this study was to evaluate the potential of solid-state fermentation (SSF) to enhance the nutritional value of oilseed cakes (OC) for poultry. To this end, firstly, we characterized the main nutrients and antinutritional factors in non-fermented OC and fermented OC, as well as in fermented and extracted OC, from where enzymatic extracts were recovered. The fermented and extracted OC represented the fermented residue obtained after SSF and extracted with distilled water. Secondly, we assessed the *in vitro* digestibility of dry matter (DM), organic matter (OM) and crude protein (CP) from these products using a poultry *in vitro* digestion model. The *in vitro* procedure used a two-stage sequential protocol simulating gastric digestion (proventriculus and gizzard - using pepsin in hydrochloric acid) and small intestinal digestion with pancreatin solution. Additionally, raw OC were supplemented with enzymatic concentrated extracts generated during SSF and their effect on nutrient digestibility was also evaluated. Solid-state fermentation exhibited a potential to reduce fiber fractions (except for lignin), decrease certain antinutritional factors, and enhance CP and mineral content in OC mixtures. However, this process concurrently diminished protein availability, through lower amino acid content, lower soluble protein, higher protein dispersibility and lower available lysine in the fermented product. The aqueous extractions led to the obtainment of a concentrated protein fraction with highly undigestible fibre. The likely effect of SSF on tannins and erucic acid content in OC should be further investigated, as SSF may not effectively reduce these antinutritional factors in the substrate, negatively affecting the overall nutritional quality of the final product. Solid-state fermented mixtures significantly increased the DM (by 12 %), OM (by 8 %), and CP (by 11 %) *in vitro* digestibility coefficients compared with non-fermented. Enzymatic supplementation did not statistically modify digestibility parameters. This study demonstrated that SSF is an effective

abbreviations: **ADFom**, acid detergent fiber; **ADL**, acid detergent lignin; **aNDFom**, neutral detergent fiber; **CP**, crude protein; **DM**, dry matter; **F**, fermented; **F_{EXT}**, fermented extracted; **HPLC**, High-Performance Liquid Chromatography; **HSD**, honestly significant difference; **LF**, liquid fermented; **NF**, non-fermented; **OC**, oilseed cakes; **OM**, organic matter; **PDI**, protein dispersibility index; **RSC**, rapeseed cake; **SAS**, Statistical Analysis System; **SBC**, soybean cake; **SFC**, sunflower cake; **SSF**, solid-state fermentation; **UV/VIS**, ultraviolet-visible.

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approach to obtain value-added products. However, it is crucial to ensure its effect on protein quality and amino acid availability is given special attention if applied to animal feed.

1. Introduction

Global consumption of animal proteins according to OECD-FAO Agricultural Outlook predictions, are expected to increase by 14 % by 2030 compared to the period of 2018–2020 (OECD/FAO, 2021). Over the last years, poultry is becoming the consumers' preference choice of animal protein supply (OECD/FAO, 2021), due to the lower price of chicken compared to other meats, its healthy fatty acid profile and its convenience of preparation. In fact, this protein source is expected to represent 41 % of all protein from meat sources by 2030 (OECD/FAO, 2021).

Maize and soybean are the most common energy and protein sources in poultry feed worldwide, respectively (Ravindran, 2013). Undoubtedly soybean cake is the highest produced oilseed cake worldwide, followed by rapeseed cake and sunflower cake (OECD/FAO, 2021). However, the fiber content of these products, particularly the amount of protein bonded in the fiber matrix (i.e. neutral detergent fiber protein), as well as the presence of antinutritional factors may reduce the availability of certain nutrients and thus limit their nutritional value when used as animal feed (Arrutia et al., 2020).

Due to the projected growth of the poultry industry, it is essential to find alternative sources of protein for feed that can reduce the pressure on natural resources and/or implement feed valorisation treatments that can enhance the nutritional value of the feed while maximizing feed efficiency and optimizing resources use. Indeed, this would contribute to moving animal feeding systems towards sustainable intensification premises; increasing production yields in ways that place far less pressure on the environment and without compromising the capacity to continue producing food in the future (Garnett et al., 2013).

Solid-state fermentation (SSF) can be used to valorize agro-industrial by-products such as brewer's spent grains, olive pomace, grape mark, vine-shoot trimmings, wheat bran, rice bran, using an eco-friendly approach with low environmental impact compared to conventional submerged fermentation (Table 1) (Zhao et al., 2017; Ferreira et al., 2020; Leite et al., 2021; Ritthibut et al., 2021; Sousa et al., 2018, 2021). In SSF, microorganisms are added to solid substrates, using them as support and as a source of nutrients, essential for their growth and development. This process occurs in the near absence of free water mimicking the conditions of the natural habitat and filamentous fungi are well-adapted microorganisms to this type of fermentation. Filamentous fungi can produce extracellular enzymes that can hydrolyse the lignocellulosic structure of cell wall polymers present in plants, decreasing the low-nutritional fiber content of these materials. Lignocellulolytic enzymes such as cellulase and xylanase can further degrade cellulose and hemicellulose polysaccharides into simple oligosaccharides chains, making fiber-associated proteins more accessible (Chukwuma et al., 2020). Additionally, the deconstruction of the lignocellulosic network may allow the release of phenolic compounds (Verduzco-Oliva and Gutierrez-Uribe, 2020). However, despite the recognized advantages of SSF to improve the chemical properties of by-products and agro-food wastes, few studies have explored their potential to enhance the nutritional value of these vegetable products for animal feeding (Santos et al., 2015; Shi et al., 2017; Ibarruri et al., 2021).

Non-invasive *in vitro* models, can be used in the laboratory to evaluate the nutritive value of feedstuffs (an example in poultry is the method described in Farhadi et al. 2019). Moreover, they can contribute to implementing the three Rs (Replacement, Reduction and Refinement) in animal research (Russell and Burch, 1959). *In vitro* digestibility methods can be employed to evaluate the digestibility of feed ingredients, the efficiency of exogenous enzymes and nutrient availability in a diet (Bedford and Classen, 1993).

In this framework, our hypothesis was that oilseed cakes (OC) exposed to SSF could change their chemical composition, produce enzymes and thus affect the nutritive value of these protein concentrates when fed to monogastric animals. Therefore, our main goal was to evaluate the potential of SSF to enhance the nutritional value of OC for poultry. Particularly, our specific objectives were firstly to characterize the main nutrients and antinutritional factors in non-fermented and fermented OC, as well as in fermented OC submitted to an extraction process from where enzymatic extracts were recovered. Secondly, to assess the *in vitro* digestibility of dry matter (DM), organic matter (OM) and crude protein (CP) from these products using a poultry *in vitro* digestion model. Finally, to determine the effect of supplementation of raw OC with enzymatic concentrated extracts generated during SSF on nutrient digestibility.

Table 1
Advantages and disadvantages of solid-state fermentation compared to conventional liquid-submerged fermentation in bioreactors.

Advantages	Disadvantages
Cost-effective	Challenges due to temperature increase; heat accumulation, oxygen, and mass transfer, pH control
Eco-friendly	
Less energy demand for heating	
Higher productivity	
Reduced wastewater with less risk of bacterial contamination	Steady aeration is difficult
Minimal degradation of enzymes	Kinetics study is still challenging

2. Materials and methods

2.1. Oilseed cakes and solid-state fermentation process

Three OC from the vegetable oils industry originally from one batch were used: sunflower cake (SFC), rapeseed cake (RSC) and soybean cake (SBC). The SFC was provided by Sorgal SA, Portugal while RSC and SBC were provided by IBEROL (Sociedade Ibérica de Oleaginosas, SARL, Portugal). Although from one batch, the samples were representative of the products processed in each site (Sousa et al., 2021). Following previous optimization studies reported by Sousa et al. (2022), OC were blended in different proportions and identified as mixture 1, NF1 (non-fermented 1), representing the combination of 1:1 (w/w) RSC:SBC, and mixture 2, NF2 (non--fermented 2), being the combination of the three OC in a ratio of 1:1:1.

The NF1 and NF2 were subjected to SSF in tray-type bioreactors, as previously described by Sousa et al. (2022) using *Aspergillus niger CECT 2915* strain. Briefly, a total amount of 400 g of dry OC mixtures was used with moisture adjusted to 75 % with distilled water (w/w, wet basis). OC were sterilized for 15 min at 121 ºC. A sterile solution of peptone composed of 1 g L^{-1} and 0.1 g L^{-1} Tween-80 was used to recover *A. niger* spores from PDA slants and to prepare an inoculum suspension with an adjusted concentration of 10^6 spores mL^{-1}. From this last solution, 80 mL was added to each tray in aseptic conditions. After inoculation trays were kept in a controlled temperature chamber for 7 days at 25 ºC. Each experiment was performed in duplicate and a control experiment without inoculation was performed in the described conditions.

After SSF, the fermented solid was extracted with water and both the solid and liquid phases were stored for further analysis. Therefore, three different fractions were obtained and identified as i) fermented (F), representing the whole fermented residue obtained after SSF; ii) fermented-extracted (F_{EXT}), representing the fermented residue obtained after SSF and extracted with distilled water in a ratio of 1:5 (w/w) solid:liquid (stored solid phase); and iii) liquid fermented (LF), liquid fraction obtained from the extraction of each combination of residues containing the enzymatic concentrated extracts (stored liquid phase). The enzymatic activity of LF has been previously characterized, with the exception of phytase (Sousa et al., 2022).Therefore, three samples were obtained from each mixture (Mix1 and Mix2): non-fermented (NF), F and F_{EXT}; resulting in six samples in total; that were subsequently used in the *in vitro* tests.

Additionally, to evaluate the effect of supplementation with enzymatic concentrated extracts obtained from SSF, raw (non-fermented) SFC, RSC, and SBC, were individually supplemented with LF extract and subjected to *in vitro* assay.

The LF extracts contained carbohydrase's (cellulase, xylanase and β-glucosidase) and protease enzymatic activity (previously characterized by Sousa et al. (2022). Due to the similarity between LF extracts form Mix1 and Mix2, LF1 (LF from Mix1) was selected to be used in this work. Table 2 shows the enzymatic characterization of LF1. Three enzymatic concentrations were evaluated, corresponding to: level 0, 0 Units per kg of raw substrate; level 1, 2194 U of cellulase, 10,018 U of xylanase, 932 U of β-glucosidase, 1207 U of protease and 500 U of phytase per kg of raw substrate and level 2, 4388 U of cellulase, 20,036 U of xylanase, 1864 U of β-glucosidase, 2414 U of protease and 1000 Units of phytase per kg of raw substrate. To achieve the final concentrations of level 2, 1.342 g of lyophilized enzymatic cocktail was dissolved in 50 mL of 0.25 M acetate buffer, pH 5.5 and 125 μL of the mixture was added to the reaction mixture. To achieve the final concentration of level 3, 250 μL of enzymatic extract was added to the reaction mixture. All samples were freeze-dried and stored at −20 °C before analyses.

Fig. 1 shows a detailed scheme of the experimental procedure used in this work.

2.2. Enzimatic activity of LF extracts

The enzymatic activity of cellulase, xylanase, β-glucosidase and protease were quantified as previously described by Sousa et al. (2022). One unit of enzyme activity was defined as the amount of enzyme required to release 1 μmol of glucose or xylose, respectively, for cellulase or xylanase, per minute at 50 °C and pH 4.8. One unit of β-glucosidase enzyme activity was defined as the amount of enzyme required to release 1 μmol of *p*-nitrophenol per minute at 50 °C and pH 4.8. One unit of protease enzyme activity was defined as the amount of enzyme required to release 1 μmol of azopeptides in 1 min at 37 °C and pH 5.0.

Phytase activity was determined following the method described by Gunashree and Venkateswaran (2015). One unit of phytase activity was defined as the amount of enzyme required to release 1 μmol of inorganic phosphorus from the substrate in 1 min at 40ºC.

Table 2
Enzymatic characterization of liquid concentrated extract from fermentation of mixture 1 (LF1).

Enzyme	Activity (U g^{-1})
Cellulase	327 ± 44
Xylanase	1493 ± 20
β-glucosidase	139 ± 20
Protease	180 ± 3
Phytase	78 ± 11

A total of three independent samples were analysed for each enzymatic activity.

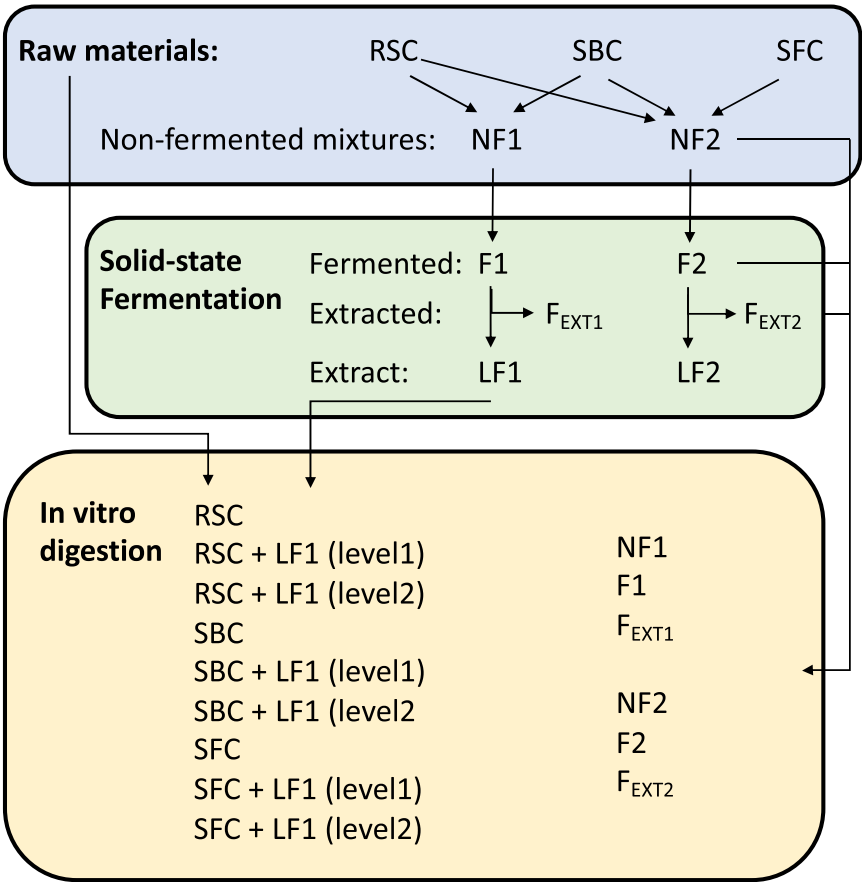

Fig. 1. Experimental design scheme. RSC: rapeseed cake; SBC: soybean cake; SFC: sunflower cake. NF: no-fermented, F: fermented; F_{EXT}: fermented and extracted; LF: liquid fermented. Level 1: 500 U phytase per kg of raw material; Level 2: 1000 U phytase per kg of raw material.

2.3. Nutrient composition

The NF, F and F_{EXT} samples from mixtures 1 and 2 were analysed in duplicate using methods of the AOAC (2019) for DM (nº 934.01) and ashes (nº 942.05). The nitrogen content was quantified using the Kjeldhal method (Chemists and Horwitz, 1975) and estimation of crude protein was obtained using a defined factor of 6.25. The neutral detergent fiber (aNDFom), acid detergent fiber (ADFom) and acid detergent lignin (ADL) fractions were analysed sequentially according to (Mertens, 2002), procedure nº 973.18 of the AOAC (2019) and Van Soest et al. (1991), respectively, with a thermo-stable α-amylase pre-treatment and expressed exclusive of residual ash, by using a nylon filter bag system (Ankom, Macedon, NY, USA). Amino acids profile was quantified using Waters High-Performance Liquid Chromatography (HPLC) with a column Waters Acc. Tag (3.9 d.i. x 150 mm). Prior to HPLC analysis, for total amino acids quantification, samples were first subjected to acid hydrolysis with HCl at 110 °C for 23 h. For quantification of sulphurated amino acids, samples were primarily oxidized with performic acid for 16 h at 4 °C, followed by acid hydrolysis with HCl at 110 °C for 23 h.

True protein content was calculated based on the difference between total Kjeldhal nitrogen and non-protein nitrogen (urea and ammoniacal nitrogen) all determined using Kjeldhal method. To obtain the value of the true protein content, the amount of nitrogen obtained by this difference was multiplied by a factor of 6.25.

2.4. Antinutritional factors and protein quality parameters

The presence and quantification of antinutritional factors was performed in each sample of NF, F and F_{EXT} from mixture 1 and 2. Phytic acid content was determined with the phytic acid/total phosphorus K-PHYT (Megazyme International, County Wicklow, Ireland) enzymatic kit. Tannins were quantified through ultraviolet-visible (UV/VIS) spectrophotometry (PerkinElmer, Waltham, MA, USA). Glucosinolates were quantified by volumetric analysis through the determination of allyl isothiocyanate by steam distillation based on BOE (1995). The presence of erucic acid was quantified using gas chromatography. Protein solubility in potassium hydroxide (KOH) was determined following the method described in Araba and Dale (1990), protein dispersibility index (PDI) and trypsin inhibitor was determined following (Society, 2000) and urease activity index was determined using a modified method of (Caskey and Knapp, 1944) based on pH increments. Determination of available lysine was performed through UV/VIS spectrophotometry

(PerkinElmer, Waltham, MA, USA).

2.5. In vitro poultry digestion model

To evaluate the *in vitro* digestibility coefficients of NF, F and F_{EXT} from SSF process (of both mixtures), as well as the raw materials supplemented with LF1 concentrated extract (at 3 levels), fifteen samples were analysed in triplicate using an *in vitro* digestion model for monogastrics (Boisen and Fernández, 1997; Pascual et al., 2000). The *in vitro* DM, OM, and CP digestibility coefficients were determined. DM (nº 934.01), ashes (nº 942.05) and CP (Kjeldahl) of the residue after digestion were analysed using methods of the AOAC (2019) as previously described.

The *in vitro* procedure used was a two-stage sequential protocol where the first stage was the gastric phase (proventriculus and gizzard) digestion using pepsin in the presence of hydrochloric acid (HCl) and the second stage the simulation of small intestinal digestion using pancreatin solution, following Farhadi et al. (2019). Prior to the gastric phase, the crop phase was simulated as described in Hirvonen et al. (2019).

Briefly, to simulate the crop phase, a sample weight equivalent to 250 mg CP (N x 6.25) was introduced in 50 mL Erlenmeyer flasks and 3 mL of phosphate-free sterile water was added. Samples were kept at 40 °C for 20 min. After incubation, 4.5 mL of freshly prepared pepsin from porcine gastric mucosa, containing 2000 U of pepsin per mL (P6887, Sigma-Aldrich) dissolved in 0.13 M HCl, was added to each flask. The pH was adjusted to 2.5 with 6 M HCl or 6 M NaOH. Flasks were placed on a shaking water bath at 140 strokes per minute for 42 min at 40 °C. At the end of this stage (gastric phase), 1.5 mL of 1 M $NaHCO_3$ containing 3 mg mL^{-1} of pancreatic solution from porcine pancreas (P7545, Sigma-Aldrich) was added to each flask. Flasks were vortexed and pH was adjusted to 6.3–6.7 with 6 M HCl or 6 M NaOH. All flasks were placed on the shaking water bath for 60 min at 40 °C. At the end of the small intestine phase, 1 mL of 100 g L^{-1} trichloroacetic acid was added to each tube. Tubes were kept at 20 °C for 30 min. Precipitated protein fractions were filtered by a Gooch crucible (pore diameter: 40–90 μm) in a cold extraction unit of fiber 1021 cold extractor, Tecator™, Denmark. Sediments were rinsed three times sequentially with water, ethanol, and acetone according to Kovitvadhi et al. (2021). Celite was used during the filtration process. Crucibles were dried at 105 °C for 24 h and recovered samples were stored at 20 °C until analysis.

Table 3
Nutrient composition of non-fermented (NF), fermented (F) and fermented-extracted (F_{EXT}) oilseed cake mixtures from sunflower cake (SFC), rapeseed cake (RSC) and soybean cake (SBC).

	Raw Oilseed cakes			Mixture 1, ratio 1:1 (RSC:SBC)			Mixture 2, ratio 1:1:1 (SFC:RSC:SBC)		
g/kg, as fed	SFC	RSC	SBC	NF1	F1	$F_{EXT}1$	NF2	F2	$F_{EXT}2$
DM	899.3	858.6	898.4	872.1	928.6	942.8	879.2	931.3	938.3
Ash	81.8	74.9	68.8	68.9	101.1	77.2	71.1	105.3	75.4
C. P.	458.8	489.7	560.0	540.9	621.4	506.6	505.9	602.1	499.2
aNDFom	366.3	413.1	154.7	281.1	220.9	337.3	314.4	233.6	324.3
ADFom	247.4	268.4	92.2	178.8	156.3	247.3	213.0	173.5	238.1
ADL	61.7	96.1	1.30	49.7	68.8	105.1	67.4	71.3	98.7
True protein	405	386	441	397	333	380	386	317	349
Ammoniacal N	0.6	< 0.5	< 0.5	< 0.5	26.8	11.8	0.5	29.3	10.7
Gross energy (cal/g)	4075	4068	4164	4116	3589	4050	4061	3619	3997
Amino acids									
Asp	40.4	26.3	68.8	41.7	36.8	32.8	42.0	36.9	30.4
Ser	20.1	15.7	44.3	20.7	18.1	16.8	20.9	18.6	20.7
Glut	83.8	65.9	93.4	80.1	63.8	51.0	79.0	45.8	53.3
Gly	27.7	23.1	27.3	25.4	23.0	21.8	22.3	19.7	23.6
His	11.5	12.3	18.9	13.6	5.6	9.2	10.2	8.1	10.5
Arg	34.4	21.6	38.9	30.8	26.0	21.5	28.0	19.1	21.0
Thr	15.6	16.0	23.4	18.7	18.0	16.0	16.0	14.3	16.0
Ala	16.6	16.0	23.7	18.4	19.0	18.7	16.7	15.6	19.0
Pro	16.3	22.1	26.5	23.2	19.0	17.1	19.4	15.0	16.8
Cys	4.6	6.8	6.2	7.0	9.6	4.7	3.7	6.3	6.5
Tyr	9.9	11.1	20.4	14.3	15.2	11.9	10.7	10.5	11.2
Val	23.8	22.8	31.6	25.1	23.2	23.6	21.4	20.4	24.3
Met	10.1	9.7	8.8	9.3	10.0	10.3	9.6	9.4	11.2
Lys	15.3	19.1	33.3	25.1	19.0	18.3	20.1	15.6	16.1
Ile	17.9	16.3	27.8	20.4	17.1	18.0	17.3	14.9	18.0
Leu	28.1	28.5	43.4	35.0	31.9	30.2	29.1	24.0	30.2
Phe	17.8	14.4	25.6	20.0	17.1	15.9	16.5	13.0	15.8

D. M.– dry matter, C- carbon, N- nitrogen, C. P.– crude protein, aNDFom- neutral detergent fiber, ADFom- acid detergent fiber, ADL- Acid detergent lignin, Asp- Aspartic acid, Ser- Serine, Glut- Glutamic acid, Gly- Glycine, His- Histidine, Arg- Arginine, Thr- Threonine, Ala- Alanine, Pro- Proline, Cys- Cysteine, Tyr- Tyrosine, Val- Valine, Met- Methionine, Lys- Lysine, Ile- Isoleucine, Leu- Leucine, Phe- Phenylalanine

2.6. Calculations and statistical analysis

Digestibility of DM, OM and CP in percentage were calculated as the difference between the initial nutrient content of each sample and the undigested nutrient in the residue, corrected for the blank, concerning the initial nutrient content and multiplied by 100.

Analysis of variance for *in vitro* digestibility and phytase activity followed by post hoc Turkey's honestly significant difference (HSD) test was carried out using (SAS, 2002). Data from NF, F and F_{EXT} were analysed using a model that included the treatment, the mixture and their interaction as fixed effects. Data from raw materials with LF were analysed using a model that included the raw material, the LF level and their interaction as fixed effects.

3. Results

3.1. Effect of solid-state fermentation on oilseed cakes characterization (nutrient composition and antinutritional factors)

3.1.1. Nutrient composition

Table 3 shows the chemical composition of the OC used in this study, as well as the mixtures created, before the SSF (NF), after the SSF (F) and after extraction of the LF extracts (F_{EXT}).

The composition of single OC and NF mixtures of OC obtained showed large variations in CP from 459 to 560 g/kg, and in fibrous fractions (aNDFom, varying from 155 to 413 g/kg; ADFom, varying from 92 to 268 g/kg and ADL, varying from 1 to 96 g/kg). SBC presented the highest CP and the lowest fiber concentrations, while RSC showed the highest concentration of fibrous fractions.

Compared to NF mixtures, F mixtures exhibited numerically higher DM, CP, ash and ADL (for F_1) contents. As regards the fermented OC obtained after the extraction of soluble compounds (F_{EXT}), these were characterized by a high concentration of fiber fractions, as soluble fractions were recovered in the LF extract which contained a concentrated cocktail of enzymes with soluble minerals, sugars, proteins, and phenolic compounds.

According to Table 3, there was a numerical decrease in almost all amino acids after SSF, except for cystine; as well as a decrease in true protein. Fermented mixtures exhibited numerically higher ammoniacal nitrogen content compared to NF. Numerically lower levels of ammoniacal nitrogen and higher levels of true protein were observed in F_{EXT} in comparison to F.

3.1.2. Antinutritional factors and protein quality

Table 4 shows the characterization of the main antinutritional factors and protein quality parameters in the tested OC mixtures. Compared to NF mixtures, F mixtures exhibited numerically higher tannins and erucic acid concentration, but remarkably lower phytic acid and glucosinolate content (except for mixture 2, where glucosinolate content was numerically higher in F than in NF mixtures).

As regards protein quality parameters from the tested samples, F mixtures exhibited numerically lower protein solubility in KOH, urease activity and lysine content after SSF in both mixtures. Reductions of lysine content ranged from 7 % to 15 % after SSF for both mixtures of OC.

On the other hand, there was a numerical increment of protein dispersibility index (PDI) in F mixtures compared to NF, except in F_{EXT}. Compared to NF mixtures, F mixtures exhibited numerically higher trypsin inhibitors.

3.2. Effect of solid-state fermentation of oilseed cakes on in vitro nutrient digestibility

Table 5 depicts the results of DM, OM and protein digestibility of NF, F and F_{EXT} of OC mixtures 1 and 2. There was a significant effect ($P < 0.05$) of the mixture and treatment on the digestibility coefficients of DM, OM, and CP, except for the mixture effect on CP digestibility. In addition, there was a significant effect ($P < 0.05$) of the interaction between mixture and treatment on DM and OM digestibility.

Average *in vitro* digestibility coefficients for DM for NF and F products ranged from 0.448 to 0.536, while F_{EXT} coefficients were

Table 4
Antinutritional factors and protein quality parameters of non-fermented, fermented and fermented-extracted oilseed cake mixtures from sunflower cake (SFC), rapeseed cake (RSC) and soybean cake (SBC) on dry matter basis.

	NF1	F1	$F_{EXT}1$	NF2	F2	F_{EXT2}
Antinutritional factors (g/kg, as fed)						
Phytic acid	29.7	n.d.	2.4	35.5	n.d.	3.8
Tannins	0.23	0.58	0.29	0.22	0.60	0.44
Glucosinolates	0.08	< 0.01	< 0.01	0.05	0.35	0.38
Erucic acid[1]	0.01	0.03	0.03	0.01	0.02	0.02
Physical chemical protein properties						
Soluble Protein (KOH)	5.31	4.95	5.15	5.69	5.2	5.03
Protein dispersibility index	1.34	2.46	1.63	2.07	2.75	1.96
Trypsin inhibitor	0.69	1.60	1.00	0.46	2.79	0.94
Urease Activity Index[2]	0.16	0.02	0.04	0.15	0.01	0.05
Available Lysine	0.18	0.17	0.16	0.15	0.13	0.14

n.d., non-determined; 1 g of erucic acid expressed in 1 kg of fatty acids; 2Units change in pH using the modified Caskey-Knapp procedure

Table 5
In vitro nutrient digestibility coefficients (dry matter-DM dig, organic matter-OM dig and crude protein- CPdig) of non-fermented, fermented and fermented-extracted oilseed cake mixtures from sunflower cake (SFC), rapeseed cake (RSC) and soybean cake (SBC).

	NF1	F1	$F_{EXT}1$	NF2	F2	$F_{EXT}2$	SEM	P-value treatment x mixture	P-value treatment (NF, F, F_{EXT})	P-value mixture (1,2)
DMdig	0.498^{b}	0.536^{a}	0.353^{d}	0.448^{c}	0.522^{ab}	0.331^{d}	0.0737	0.029	<0.001	<0.001
OMdig	0.814^{b}	0.860^{a}	0.727^{d}	0.772^{c}	0.857^{a}	0.716^{d}	0.0736	0.001	<0.001	<0.001
CPdig	0.644^{bc}	0.675^{ab}	0.495^{d}	0.629^{c}	0.696^{a}	0.480^{d}	0.0865	0.084	<0.001	0.703

SEM, standard error of the mean. Results represent the average of five independent batches. Coefficients are expressed as a ratio of 1. Values with equal letters are not statistically significant different (Tukey test; $P < 0.05$). Statistical analysis is related to each digestibility parameter.
Treatments: NF, non-fermented mixture of oilseed cakes; F, mixture of oilseed cakes fermented by *A. niger*; F_{EXT}, mixture of oilseed cakes fermented with *A. niger* followed by an aqueous extraction. Condition 1, rapeseed cake + soybean cake (1:1); Condition 2, rapeseed cake + soybean cake + sunflower cake (1:1:1).

around 0.331–0.353. The OM digestibility coefficients varied from 0.716 % to 0.860 % for all products. The crude protein digestibility coefficients for NF and F mixtures differed from 0.629 to 0.696 while for F_{EXT} they were around 0.480–0.495.

The DM and OM *in vitro* digestibility coefficients were significantly ($P < 0.05$) higher in F mixtures compared to NF and F_{EXT}, following the same pattern for both OC mixtures. Higher increases of DM and OM coefficients were observed for the ternary combination of OC (mixture 2, composed of 1:1:1 ratio of all three OC).

3.3. Effect of supplementation with enzymatic concentrated extracts generated during solid-state fermentation, on in vitro nutrient digestibility coefficients of oilseed cakes

Table 6 depicts the results of the *in vitro* digestion coefficients of NF OC with different concentrations of enzymatic supplementation.

Results show the supplementation with enzymes did not significantly ($P < 0.05$) affect *in vitro* nutrient digestibility coefficients in our work.

4. Discussion

The nutritional properties of single OC (SFC, RSC and SBC) used during this study were within the standard parameters recommended for animal feed formulations according to (INRAE et al., 2022), and similar to that previously reported by Sousa et al. (2021) for these same raw materials.

Although data in Table 3 and Table 4 are exploratory in nature, aiming to investigate potential changes and trends, rather than confirm statistically significant differences between treatments; our data suggest the SSF has potential to modify OC nutrient composition. These changes could be expected and attributable to the inherent fungi hydrolytic process. The production of microbial protein, generally referred to as single cell protein during SSF by *Aspergillus niger* is one of the factors that may have contributed to the potential increase of CP (Aggelopoulos et al., 2014; Canedo et al., 2016; Sousa et al., 2018). During SSF, however, the production and release of proteinaceous compounds such as enzymes and the production of nonprotein compounds including: ammonia, amino sugars such as glutamine and chitin, formation of complexes such as phenol-protein, protein-lignin or protein-chitin can also occur (Odum et al., 1979; Terefe et al., 2021). Also, during fermentation microorganisms use carbohydrates as carbon source to support their growth affecting the C/N ratio leading to a fermented product with concentrated nitrogen resulting in higher levels of protein in the total mass (Nasseri et al., 2011). The numerical high values of ADL observed after SSF could be the result of the degradation of fibrous compounds namely cellulose and hemicellulose present in OC matrix, except for lignin, which is the most undigestible fraction and recalcitrant to degradation. The numerically higher concentrations of minerals observed after SSF can be explained due to the metabolic activity of the microorganism during the fermentation process, attributed to fungal biomineralization, as their activity is known to have effects on the transformation of metals and minerals. Additionally, the decrease of fibers fraction may have resulted in a concentration of minerals.

The amino acid profile of OC is a key relevant variable for determining its nutritional value when it is intended to be used in animal feed. Insufficient or unbalanced levels of essential amino acids can limit the growth and performance of poultry (Vieira et al., 2004). Birds need adjusted amino acid levels that are ideally combined (using the ideal protein concept, expressed relative to lysine (Emmert and Baker, 1997) to meet the requirements of each amino acid without deficit or excess. Methionine followed by lysine are the first limiting amino acids in most practical poultry diets. Therefore, the implications of the amino acid profile in OC fed to poultry can be significant for poultry production and nutrition. Besides incomplete or imbalanced amino acid profiles can lead to suboptimal growth rates and negatively influence feed conversion, when additional synthetic amino acids may be required in deficient poultry diets, this may increase the cost of feed production. Furthermore, amino acids also play an essential role in the overall health and immune response of poultry. Inadequate amino acids can make birds more susceptible to diseases and stress (Beski et al., 2015).

Our results highlight a potential decline in amino acid values after SSF in OC. Several amino acids are involved in enzymatic reactions and protein synthesis and may have contributed to this decrease. Additionally, the conversion of true protein into ammoniacal nitrogen could partly explain these differences. During SSF, transformation of true protein into nonprotein compounds such as ammoniacal nitrogen has been reported (Farkhoy et al., 2012). With the aqueous extraction, soluble compounds of the lignocellulosic

Table 6
Dry matter (DM) digestibility, organic matter (OM) digestibility and crude protein (CP) digestibility of non-fermented oilseed cakes with three levels of external enzyme supplementation.

	SFC + no enzyme	SFC + enzyme level 1	SFC + enzyme level 2	RSC + no enzyme	RSC + enzyme level 1	RSC + enzyme level 2	SBC + no enzyme	SBC + enzyme level 1	SBC + enzyme level 2	SEM	P-value treatment x mixture	P-value treatment (NF, F, F_{EXT})	P-value mixture (1,2)
DMdig	0.440^{b}	0.430^{b}	0.445^{b}	0.327^{c}	0.325^{c}	0.318^{c}	0.620^{a}	0.616^{a}	0.646^{a}	0.0142	0.544	<0.001	0.684
OMdig	0.764^{b}	0.761^{b}	0.771^{b}	0.665^{c}	0.655^{c}	0.661^{c}	0.903^{a}	0.896^{a}	0.916^{a}	0.0083	0.239	<0.001	0.833
CPdig	0.719^{a}	0.680^{a}	0.680^{a}	0.430^{b}	0.416^{b}	0.402^{b}	0.729^{a}	0.731^{a}	0.768^{a}	0.0199	0.308	<0.001	0.585

SFC, sunflower cake; RSC, rapeseed cake; SBC, soybean cake; enzyme level 1, supplementation with 2194 U of cellulase, 10,018 U of xylanase, 932 U of α-glucosidase, 1207 U of protease and 500 U of phytase per kg of raw substrate; enzyme level 2, supplementation with 4388 U of cellulase, 20,036 U of xylanase, 1864 U of α-glucosidase, 2414 U of protease and 1000 Units of phytase per kg of raw substrate; SEM, standard error of the mean. Results represent the average of six independent experiments. Values with equal letters are not statistically significant different (Tukey test; $P < 0.05$). Statistical analysis is related to each digestibility parameter.

matrix of OC as well as nonprotein compounds were eluted resulting in a concentrated F_{EXT} with lower levels of ammoniacal nitrogen and higher levels of true protein in comparison to F.

The presence of antinutritional factors in OC is one of the main drawbacks that limit their nutritional value as animal feed (Woyengo et al., 2017). Besides antinutritional factors, physical-chemical protein properties (e.g., protein solubility, urease activity and reactive lysine, amongst others) are valuable indicators of the protein quality and availability of the amino acids contained in them for the animals (Schoenlechner et al., 2008). Phosphorus (P) is an essential and limiting nutrient in poultry feed and phytic acid or phytate is the main storage form of organic P in plant seeds. As poultry do not generate phytase that can hydrolyse phytic acid, the P contained in the phytic acid molecule cannot be utilized by the animals (Javadi et al., 2022). Additionally, phytic acid is a strong chelation agent that binds to important mineral cofactors influencing the activity of digestive proteins (Gilani et al., 2012). The potential of SSF to decrease phytic acid concentrations has been previously reported (Hu et al., 2019; Olukomaiya et al., 2020) and can be attributed to the production of the extracellular enzyme phytase by *A. niger*. The phytase produced over the course of SSF potentially acts over phytase engaging in a series of dephosphorylation steps resulting in a more accessible form of phosphorus that can be absorbed by poultry (Dersjant-Li et al., 2015).

Tannins are naturally occurring groups of phenolic compounds, considerable undesirable in animal nutrition as they precipitate proteins, may inhibit digestive enzymes, limits minerals and vitamins utilization and can negatively affect feed palatability (Ram et al., 2020). However, the presence of small concentrations of tannins has been reported to improve the overall health status, nutrition, and animal performance in monogastrics (Biagi et al., 2010; Starčević et al., 2015). These positive effects are related to the promotion of intestinal ecosystem health through tannins anti-oxidant, anti-microbial and anti-inflammatory potential (Huang et al., 2018). The increment of tannins during SSF may be related to the delignification of lignocellulosic fractions of OC. Bound phenolic compounds can be connected to structures of vegetable cell walls such as hemicellulose or lignin and can be released by the action of extracellular enzymes produced during SSF. The release of phenolic compounds from the OC matrix, using the same OC has been previously described by Sousa et al. (2021). Additionally, tannins are water soluble compounds and for that reason their concentration could have been potentially decreased after SSF in F_{EXT}.

Glucosinolates and erucic acid are characteristic antinutritional factors of RSC. The main toxic compounds are glucosinolates and their degradation products. High glucosinolates ingestion in hens increased mortality and lowered egg production as well as egg weights (Tripathi and Mishra, 2007). On the other hand, the main effects observed when feeding poultry with diets containing high erucic acid were growth retardation and cardiac lipidosis (Knutsen et al., 2016). The observed trend to decrease glucosinolates in mixture 1 may be related to the capacity of *A. niger* to produce a myriad of extracellular enzymes able to degrade these compounds (Shi et al., 2016). On the other hand, despite the observed trend to increase erucic acid after SSF, the concentration of this parameter in these OC was considerably low representing less than 0.03 g/kg of total fatty acids. According to the European Food Safety Authority, the lowest level with adverse observed effects identified for liver toxicity in poultry was after exposure to erucic acid in a concentration of 20 mg kg^{-1} by weight, per day (Knutsen et al., 2016).

As regards the physical-chemical properties of proteins, protein solubility in KOH and PDI (means of comparing the solubility of a protein in water) are measurements commonly employed when using protein concentrates to evaluate the extent of thermal processing (Salazar-Villanea et al., 2016). Thermal processing is often used to decrease the content of antinutritional factors in OC. However, while under-processing treatments may not affect antinutritional factors concentration, overprocessing may affect protein stability, particularly available lysine. The KOH solubility ranging from 70 % to 85 % is generally regarded as ideal for soybean meal reflecting a good quality protein (Heuzé et al., 2020). The solubility of proteins could be altered during the heating, especially during the sterilization of OC leading to a denaturation of a small portion of proteins present in OC mixtures. The extraction of soluble compounds namely nonprotein N compounds after SSF could explain the numerically lower PDI of F_{EXT} mixtures which, compared with the respective F mixtures was approximately 1.5-fold. A potential increase in PDI after SSF could be attributed to the presence of nonprotein compounds that increase PDI.

Trypsin inhibitors (one of the most important antinutritional factor present in OC) are mainly found in SBC and they inhibit the action of proteolytic enzymes in the digestive tract reducing trypsin and chymotrypsin activity and consequently protein digestion (Erdaw et al., 2016). Our results provided initial insights into the potential effect of SSF on trypsin inhibitors. There was a numerical trend to increase trypsin inhibitors in F compared to NF mixtures. However, according to Chen et al. (2020), the observed content of 2, 79 g/kg for F_2 is within the recent indicators of analysed SBM in European countries. Trypsin inhibitors are water soluble compounds and this can explain the potential reduction of this parameter for F_{EXT} (Avilés-Gaxiola et al., 2018).

Although the presence of urea does not affect poultry productivity, urease enzyme can be inactivated by heat (Ibáñez et al., 2020). Therefore, urease activity can indirectly provide information on over or under-processing of the products and thus on the potential antinutritional factors present other than urea (such as trypsin inhibitors). Urease activities in excess of a 0.15 units suggest under processing (and thus potentially trypsin inhibitors remaining in the meal), while activities of less than 0.05 units indicated overprocessing (Georgia, 2022). An excess of heat, can increase the incidence of Maillard reactions, reducing the digestibility of amino acids like lysine and the nutritive value of OC (Ibáñez et al., 2020).

Lysine is an essential amino acid and is the second limiting amino acid, after methionine, in poultry diets (Baker and Han, 1994). This amino acid is used as reference in feed formulations due to its importance in body protein deposition (Tesseraud et al., 1996). Shi et al. (2021) observed a decrease of lysine content after SSF of drumstick leaf flour with a microbial consortium composed by *A. niger*, *Candida utilis* and *Bacillus subtilis*. The inoculated microorganism may have used lysine for metabolic activity leading to lower concentrations in the fermented substrate compared to raw OC. Special attention should be directed towards understanding the mechanisms underlying the potential negative effect of SSF on lysine. Further investigations on the differential metabolic use of this essential amino acid by fungal species could help elucidate how to overcome this aspect, particularly when SSF is intended to valorize

agro-industrial by-products for animal feed. Further advancements in fermentation technology and optimization of process conditions may help address some of these limitations.

Overall, our data point out a relevant potential of SSF to reduce fiber content in OC and enhance CP and mineral content. However, it should be noted that this process may concurrently diminish protein availability, through lower amino acid content, lower soluble protein, higher protein dispersibility, and lower available lysine in the fermented product. Furthermore, the likely effect of SSF on antinutritional factors in OC should be further investigated, to prevent a decrease in the overall nutritional quality of the final product.

One of the limitations of our study resides in the use of mixed OC. As individual OC were not fermented, we cannot elucidate where, or from which OC the effect is coming from. However, the selection of the mixture of OC was based on previous experiments of substrate optimization where we used statistical software to maximize the crude protein and enzymes and minimize the fibre content (Sousa et al., 2022). The optimum combination of OC were the ones used in this work.

The inclusion of SFC in mixture 2 and consequently the lower proportion of SBC compared to mixture 1 led to lower digestibility values of NF mixture 2. Mixture 2 included 2/3 of highly fibrous protein concentrates (SFC and RSC) that impaired the overall digestibility of this mixture compared to mixture 1. From the three OC tested in this study, SFC has been reported as the one with a higher content of undigestible fiber, followed by RSC and SBC (Lannuzel et al., 2022).

Through SSF it was possible to increase the DM digestibility of F mixtures 1 and 2 by 3.77 and 7.38 percentage points compared to the respective non-fermented mixtures. The increments of OM digestibility of fermented OC were lower compared to the DM digestibility but still statistically significant. The increase in OM was equal to 4.61 percentage points for F_1 compared to non-fermented mixture 1; and 8.50 percentage points for F_2 compared to non-fermented mixture 2. The OM fraction does not include minerals and inorganic compounds which in the *in vitro* systems are not retained while in the *in vivo* experiments these compounds could be absorbed by the animal.

In contrast to F mixtures, F_{EXT} showed lower digestibility values as a consequence of the aqueous extraction. This extraction removed fermentable carbohydrates and other soluble organic compounds (proteins, phenolic compounds, organic acids) from fermented OC obtained by the enzymatic hydrolysis of structural carbohydrates (cellulose and hemicellulose) and protein fractions of OC. As a consequence of the extraction process, F_{EXT} mixtures showed a concentrated fraction of highly undigestible fiber (lignin) compared to NF mixtures and this fraction is not degraded by endogenous digestive enzymes (Knudsen, 2001; Röhe and Zentek, 2021). DM digestibility of F_{EXT} in mixtures 1 and 2 was respectively 1.41 and 1.35-fold lower than the corresponding non-fermented mixtures. The reduction of OM digestibility for F_{EXT} was 1.12-fold for mixture 1 and 1.08-fold for mixture 2 compared to the corresponding NF mixtures.

The results of protein digestibility showed an increase in F mixtures compared to the corresponding non-fermented mixtures. Increases of 3.16 and 6.67 percentage points were observed for mixtures 1 and 2, respectively; although only F_2 showed a statistically significant increase compared to NF mixture. Also, the fermented ternary combination of OC (F_2) achieved protein digestibility values significantly higher than NF mixture 1 which had SBC concentration 1.5-fold higher than non-fermented mixture 2. The aqueous extraction resulted in lower digestibility coefficients of F_{EXT} mixtures because of the extraction of nonprotein N compounds obtained from the action of proteolytic enzymes during SSF. Reductions around 1.3-fold were observed for F_{EXT} mixtures. The inclusion of SFC in the ratio of 1:1:1 with RSC and SBC did not result in statistically significant differences in protein digestibility achieving similar values between the corresponding mixtures of NF, F and F_{EXT}.

Increase in nutrient *in vitro* digestibility coefficients has been reported for SSF products in broilers in the literature. Although in *in vivo* conditions, Alshelmani et al. (2017) reported an increase of CP digestibility from 58 % to 62 % after SSF of palm kernel cake with *Paenibacillus polymyxa in broilers*. Additionally, Hakim et al. (2020) also reported an increase in CP digestibility from 46 % to 60 % after SSF of palm kernel cake, using *Weisella confuse* in broilers, as well.

5. Conclusions

Solid-state fermentation exhibited a potential to reduce fiber fractions (except for lignin), decrease certain antinutritional factors, and enhance crude protein and mineral content in oilseed cake mixtures. However, this process concurrently diminished protein availability, through lower amino acid content, lower soluble protein, higher protein dispersibility and lower available lysine in the fermented product. This was attributed to a transformation of true protein into nonprotein compounds such as ammoniacal nitrogen and might vary depending on fungal species used. The aqueous extractions led to the obtainment of a concentrated protein fraction with highly undigestible fibre. The likely effect of SSF on tannins and erucic acid content in oilseed cake should be further investigated, as solid-state fermentation may not effectively reduce these antinutritional factors in the substrate, negatively affecting the overall nutritional quality of the final product. The *in vitro* digestibility was successfully reproduced, and solid-state fermentation mixtures significantly increased the dry matter, organic matter, and crude protein digestibility coefficients compared with non-fermented. Both fermented mixtures achieved similar digestibility values despite the incorporation of a highly fibrous oilseed cake (sunflower cake) in mixture 2. Enzymatic supplementation in different levels did not affect digestibility parameters.

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Author statement

The authors have nothing to declare.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

References

Aggelopoulos, T., Katsieris, K., Bekatorou, A., Pandey, A., Banat, I.M., Koutinas, A.A., 2014. Solid state fermentation of food waste mixtures for single cell protein, aroma volatiles and fat production. Food Chem. 145, 710–716. https://doi.org/10.1016/j.foodchem.2013.07.105.

Alshelmani, M.I., Loh, T.C., Foo, H.L., Sazili, A.Q., Lau, W.H., 2017. Effect of solid state fermentation on nutrient content and ileal amino acids digestibility of palm kernel cake in broiler chickens. Indian J. Anim. Sci. 87, 1135–1140.

AOAC, 2019. Official Methods of Analysis of the Association of Official Analytical Chemists, 21st Editi. ed. Washington DC, USA.

Araba, M., Dale, N.M., 1990. Evaluation of protein solubility as an indicator of overprocessing soybean meal. Poult. Sci. 69, 76–83. https://doi.org/10.3382/ps.0690076.

Arrutia, F., Binner, E., Williams, P., Waldron, K.W., 2020. Oilseeds beyond oil: Press cakes and meals supplying global protein requirements. Trends Food Sci. Technol. 100, 88–102. https://doi.org/10.1016/j.tifs.2020.03.044.

Avilés-Gaxiola, S., Chuck-Hernández, C., Serna Saldívar, S.O., 2018. Inactivation methods of trypsin inhibitor in legumes: a review. J. Food Sci. 83, 17–29. https://doi.org/10.1111/1750-3841.13985.

Baker, D.H., Han, Y., 1994. Ideal amino acid profile for chicks during the first three weeks posthatching. Poult. Sci. 73, 1441–1447. https://doi.org/10.3382/ps.0731441.

Bedford, M.R., Classen, H.L., 1993. An in vitro assay for prediction of broiler intestinal viscosity and growth when fed rye-based diets in the presence of exogenous enzymes. Poult. Sci. 72, 137–143. https://doi.org/10.3382/ps.0720137.

Beski, S.S.M., Swick, R.A., Iji, P.A., 2015. Specialized protein products in broiler chicken nutrition: A review. Anim. Nutr. 1, 47–53.

Biagi, G., Cipollini, I., Paulicks, B.R., Roth, F.X., 2010. Effect of tannins on growth performance and intestinal ecosystem in weaned piglets. Arch. Anim. Nutr. 64, 121–135. https://doi.org/10.1080/17450390903461584.

BOE, 1995. Royal Decret 2257/1994 for which it is aproved the official analysis methods for animal feed and first prime matters (Real Decreto 2257/1994 por el que se aprueba los métodos oficiales de análisis de piensos o alimentos para animales y sus primeras materias). BOE 52, 6.

Boisen, S., Fernández, J.A., 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Anim. Feed Sci. Technol. 68, 277–286. https://doi.org/10.1016/S0377-8401(97)00058-8.

Canedo, M.S., de Paula, F.G., da Silva, F.A., Vendruscolo, F., 2016. Protein enrichment of brewery spent grain from Rhizopus oligosporus by solid-state fermentation. Bioprocess Biosyst. Eng. 39, 1105–1113. https://doi.org/10.1007/s00449-016-1587-8.

Caskey, C.D.J., Knapp, F.C., 1944. Method for detecting inadequately heated soybean oil meal. Ind. Eng. Chem. Anal. Ed. 16, 640–641. https://doi.org/10.1021/i560134a020.

Chemists, A. of O.A., Horwitz, W., 1975. Official methods of analysis. Association of Official Analytical Chemists Washington, DC, USA.

Chen, J., Wedekind, K., Escobar, J., Vazquez-Añón, M., 2020. Trypsin inhibitor and urease activity of soybean meal products from different countries and impact of trypsin inhibitor on ileal amino acid digestibility in pig. J. Am. Oil Chem. Soc. 97, 1151–1163. https://doi.org/10.1002/aocs.12394.

Chukwuma, O.B., Rafatullah, M., Tajarudin, H.A., Ismail, N., 2020. Lignocellulolytic enzymes in biotechnological and industrial processes: a review. Sustainability 12, 7282.

Dersjant-Li, Y., Awati, A., Schulze, H., Partridge, G., 2015. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. J. Sci. Food Agric. 95, 878–896.

Emmert, J.L., Baker, D.H., 1997. Use of the ideal protein concept for precision formulation of amino acid levels in broiler diets. J. Appl. Poult. Res. 6, 462–470.

Erdaw, M.M., Bhuiyan, M.M., Iji, P.A., 2016. Enhancing the nutritional value of soybeans for poultry through supplementation with new-generation feed enzymes. Worlds Poult. Sci. J. 72, 307–322. https://doi.org/10.1017/S0043933916000271.

Farhadi, D., Karimi, A., Sadeghi, A.A., Rostamzadeh, J., Bedford, M.R., 2019. Effect of a high dose of exogenous phytase and supplementary myo-inositol on mineral solubility of broiler digesta and diets subjected to in vitro digestion assay. Poult. Sci. 98, 3870–3883. https://doi.org/10.3382/ps/pez104.

Farkhoy, M., Modirsanei, M., Ghavidel, O., Sadegh, M., Jafarnejad, S., 2012. Evaluation of protein concentration and limiting amino acids including lysine and met + cys in prestarter diet on performance of broilers. Vet. Med. Int. vol. 2012, 394189 https://doi.org/10.1155/2012/394189.

Ferreira, M., Fernandes, H., Peres, H., Oliva-Teles, A., Belo, I., Salgado, J.M., 2020. Bio-enrichment of oilseed cakes by Mortierella alpina under solid-state fermentation. LWT 134, 109981. https://doi.org/10.1016/j.lwt.2020.109981.

Garnett, T., Appleby, M.C., Balmford, A., Bateman, I.J., Benton, T.G., Bloomer, P., Burlingame, B., Dawkins, M., Dolan, L., Fraser, D., 2013. Sustainable intensification in agriculture: premises and policies, 341 Science 80, 33–34. https://doi.org/10.1126/science.123448.

Georgia, U. of, 2022. Quality Control [WWW Document]. URL ⟨https://poultry.caes.uga.edu/extension/poultry-nutrition/soybeans/quality-control.html⟩ (accessed 8.19.22).

Gilani, G.S., Xiao, C.W., Cockell, K.A., 2012. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. Br. J. Nutr. 108, S315–S332. https://doi.org/10.1017/S0007114512002371.

Gunashree, B.S., Venkateswaran, G., 2015. Extracellular phytase from Aspergillus niger CFR 335: purification and characterization. J. Food Sci. Technol. 52, 4558–4564.

Hakim, A.H., Zulkifli, I., Soleimani Farjam, A., Awad, E.A., Abdullah, N., Chen, W.L., Mohamad, R., 2020. Passage time, apparent metabolisable energy and ileal amino acids digestibility of treated palm kernel cake in broilers under the hot and humid tropical climate. Ital. J. Anim. Sci. 19, 194–202. https://doi.org/10.1080/1828051X.2020.1712266.

Heuzé, V., Tran, G., Kaushik, S., 2020. Soybean meal. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO. [WWW Document]. URL https://feedipedia.org/node/674 (accessed 6.20.22).

Hirvonen, J., Liljavirta, J., Saarinen, M.T., Lehtinen, M.J., Ahonen, I., Nurminen, P., 2019. Effect of phytase on in vitro hydrolysis of phytate and the formation of myo-inositol phosphate esters in various feed materials. J. Agric. Food Chem. 67, 11396–11402. https://doi.org/10.1021/acs.jafc.9b03919.
Hu, Y., Piao, C., Chen, Y., Zhou, Y., Wang, D., Yu, H., Xu, B., 2019. Soybean residue (okara) fermentation with the yeast Kluyveromyces marxianus. Food Biosci. 31, 100439 https://doi.org/10.1016/j.fbio.2019.100439.
Huang, Q., Liu, X., Zhao, G., Hu, T., Wang, Y., 2018. Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Anim. Nutr. 4, 137–150. https://doi.org/10.1016/j.aninu.2017.09.004.
Ibáñez, M.A., de Blas, C., Cámara, L., Mateos, G.G., 2020. Chemical composition, protein quality and nutritive value of commercial soybean meals produced from beans from different countries: A meta-analytical study. Anim. Feed Sci. Technol. 267, 114531 https://doi.org/10.1016/j.anifeedsci.2020.114531.
Ibarruri, J., Goiri, I., Cebrián, M., García-Rodríguez, A., 2021. Solid state fermentation as a tool to stabilize and improve nutritive value of fruit and vegetable discards: effect on nutritional composition, in vitro ruminal fermentation and organic matter digestibility. Animals 11, 1653.
INRAE, CIRAD, AFZ, 2022. Composition and nutritive values of feeds for cattle, sheep, goats, pigs, poultry, rabbits, horses and salmonids [WWW Document]. URL https://www.feedtables.com (accessed 5.20.22).
Knudsen, K.E.B., 2001. The nutritional significance of "dietary fibre" analysis. Anim. Feed Sci. Technol. 90, 3–20. https://doi.org/10.1016/S0377-8401(01)00193-6.
Knutsen, H.K., Alexander, J., Barregård, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., Dinovi, M., Edler, L., Grasl-Kraupp, B., 2016. Erucic acid in feed and food. EFSA J. 14, e04593 https://doi.org/10.2903/j.efsa.2016.4593.
Kovitvadhi, A., Chundang, P., Pliantiangtam, N., Thongprajukaew, K., Tirawattanawanich, C., Suwanasopee, T., Koonawootrittriron, S., 2021. Screening of in vitro nutrient digestibility coefficients of selected insect meals in broiler chickens, black-meat chickens and quails. J. Anim. Physiol. Anim. Nutr. (Berl.). 105, 305–315. https://doi.org/10.1111/jpn.13451.
Lannuzel, C., Smith, A., Mary, A.L., Della Pia, E.A., Kabel, M.A., de Vries, S., 2022. Improving fiber utilization from rapeseed and sunflower seed meals to substitute soybean meal in pig and chicken diets: A review. Anim. Feed Sci. Technol., 115213 https://doi.org/10.1016/j.anifeedsci.2022.115213.
Leite, P., Belo, I., Salgado, J.M., 2021. Co-management of agro-industrial wastes by solid-state fermentation for the production of bioactive compounds. Ind. Crops Prod. 172, 113990 https://doi.org/10.1016/j.indcrop.2021.113990.
Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85, 1217–1240.
Nasseri, A.T., Rasoul-Amini, S., Morowvat, M.H., Ghasemi, Y., 2011. Single cell protein: production and process. Am. J. Food Technol. 6, 103–116. https://doi.org/10.3923/ajft.2011.103.116.
Odum, W.E., Kirk, P.W., Zieman, J.C., 1979. Non-protein nitrogen compounds associated with particles of vascular plant detritus. Oikos Vol. 32 (No. 3), 363–367.
OECD/FAO, 2021. OECD-FAO Agricultural Outlook 2021-2030, OECD-FAO Agricultural Outlook. OECD, Paris, France. https://doi.org/10.1787/19428846-en.
Olukomaiya, O.O., Adiamo, O.Q., Fernando, W.C., Mereddy, R., Li, X., Sultanbawa, Y., 2020. Effect of solid-state fermentation on proximate composition, anti-nutritional factor, microbiological and functional properties of lupin flour. Food Chem. 315, 126238 https://doi.org/10.1016/j.foodchem.2020.126238.
Pascual, J.J., Fernández, C., Díaz, J.R., Garcés, C., Rubert-Alemán, J., 2000. Voluntary intake and in vivo digestibility of different date-palm fractions by Murciano-Granadina (Capra hircus). J. Arid Environ. 45, 183–189. https://doi.org/10.1006/jare.1999.0622.
Ram, S., Narwal, S., Gupta, O.P., Pandey, V., Singh, G.P., 2020. Anti-nutritional factors and bioavailability: Approaches, challenges, and opportunities. in: Wheat and Barley Grain Biofortification. Elsevier, Karnal, Haryana, India, pp. 101–128. https://doi.org/10.1016/B978-0-12-818444-8.00004-3.
Ravindran, V., 2013. Main ingredients used in poultry feed formulations. Poult. Dev. Rev. FAO 67–69. Palmerston North, New Zealand.
Ritthibut, N., Oh, S.-J., Lim, S.-T., 2021. Enhancement of bioactivity of rice bran by solid-state fermentation with Aspergillus strains. LWT 135, 110273.
Röhe, I., Zentek, J., 2021. Lignocellulose as an insoluble fiber source in poultry nutrition: a review. J. Anim. Sci. Biotechnol. 12 (1), 15. https://doi.org/10.1186/s40104-021-00594-y.
Russell, W.M.S., Burch, R.L., 1959. The principles of humane experimental technique. Methuen. Springfield, Illinois.
Salazar-Villanea, S., Bruininx, E.M.A.M., Gruppen, H., Hendriks, W.H., Carré, P., Quinsac, A., van der Poel, A.F.B., 2016. Physical and chemical changes of rapeseed meal proteins during toasting and their effects on in vitro digestibility. J. Anim. Sci. Biotechnol. 7 (1), 11. https://doi.org/10.1186/s40104-016-0120-x.
Santos, T.C.Do, Diniz, G.A., Brito, A.R., De, Pires, A.J.V., Franco, M., 2015. Effect of solid state fermentation on nutritional content and evaluation of degradability in cactus pear. Rev. Caatinga 28, 248–254. https://doi.org/10.1590/1983-21252015v28n328rc.
SAS, 2002. Statistical Analysis Systems Version 9.1., North Carolina, USA.
Schoenlechner, R., Siebenhandl, S., Berghofer, E., 2008. 7 - Pseudocereals, in: Arendt, E.K., Dal Bello, F.B.T.-G.-F.C.P. and B. (Eds.), Food Science and Technology. Academic Press, San Diego, USA pp. 149–VI. https://doi.org/https://doi.org/10.1016/B978-012373739-7.50009-5.
Shi, C., He, J., Yu, J., Yu, B., Mao, X., Zheng, P., Huang, Z., Chen, D., 2016. Physicochemical properties analysis and secretome of Aspergillus niger in fermented rapeseed meal. PLoS One 11, e0153230. https://doi.org/10.1371/journal.pone.0153230.
Shi, C., Zhang, Y., Lu, Z., Wang, Y., 2017. Solid-state fermentation of corn-soybean meal mixed feed with Bacillus subtilis and Enterococcus faecium for degrading antinutritional factors and enhancing nutritional value. J. Anim. Sci. Biotechnol. 8 (1), 9.
Shi, H., Yang, E., Li, Y., Chen, X., Zhang, J., 2021. Effect of solid-state fermentation on nutritional quality of leaf flour of the drumstick tree (Moringa oleifera Lam.). Front. Bioeng. Biotechnol. 9, 267. https://doi.org/10.3389/fbioe.2021.626628.
Society, A.O.C., 2000. Official Methods and Recommended Practices of the AOCS, 5th ed, Analytical methods. American Oil Chemists' Society, Urbana, IL, USA.
Sousa, D., Venâncio, A., Belo, I., Salgado, J.M., 2018. Mediterranean agro-industrial wastes as valuable substrates for lignocellulolytic enzymes and protein production by solid-state fermentation. J. Sci. Food Agric. 98, 5248–5256. https://doi.org/10.1002/jsfa.9063.
Sousa, D., Salgado, J.M., Cambra-López, M., Dias, A.C.P., Belo, I., 2021. Degradation of lignocellulosic matrix of oilseed cakes by solid state fermentation: fungi screening for enzymes production and antioxidants release. J. Sci. Food Agric. https://doi.org/10.1002/jsfa.11490.
Sousa, D., Salgado, J.M., Cambra-López, M., Dias, A., Belo, I., 2022. Biotechnological valorization of oilseed cakes: substrate optimization by simplex centroid mixture design and scale-up to tray bioreactor. Biofuels, Bioprod. Bioref. https://doi.org/10.1002/bbb.2428.
Starčević, K., Krstulović, L., Brozić, D., Maurić, M., Stojević, Z., Mikulec, Ž., Bajić, M., Mašek, T., 2015. Production performance, meat composition and oxidative susceptibility in broiler chicken fed with different phenolic compounds. J. Sci. Food Agric. 95, 1172–1178. https://doi.org/10.1002/jsfa.6805.
Terefe, Z.K., Omwamba, M.N., Nduko, J.M., 2021. Effect of solid state fermentation on proximate composition, antinutritional factors and in vitro protein digestibility of maize flour. Food Sci. Nutr. 9, 6343–6352. https://doi.org/10.1002/fsn3.259.
Tesseraud, S., Maaa, N., Peresson, R., Chagneau, A.M., 1996. Relative responses of protein turnover in three different skeletal muscles to dietary lysine deficiency in chicks. Br. Poult. Sci. 37, 641–650. https://doi.org/10.1080/00071669608417893.
Tripathi, M.K., Mishra, A.S., 2007. Glucosinolates in animal nutrition: A review. Anim. Feed Sci. Technol. 132, 1–27. https://doi.org/10.1016/j.anifeedsci.2006.03.003.
Van Soest, P.J., Robertson, J.B., Lewis, B. a, 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.
Verduzco-Oliva, R., Gutierrez-Uribe, J.A., 2020. Beyond enzyme production: solid state fermentation (SSF) as an alternative approach to produce antioxidant polysaccharides. Sustainability 12, 495. https://doi.org/10.3390/su12020495.
Vieira, S.L., Lemme, A., Goldenberg, D.B., Brugalli, I., 2004. Responses of growing broilers to diets with increased sulfur amino acids to lysine ratios at two dietary protein levels. Poult. Sci. 83, 1307–1313.
Woyengo, T.A., Beltranena, E., Zijlstra, R.T., 2017. Effect of anti-nutritional factors of oilseed co-products on feed intake of pigs and poultry. Anim. Feed Sci. Technol. 233, 76–86. https://doi.org/10.1016/j.anifeedsci.2016.05.006.
Zhao, H.-M., Guo, X.-N., Zhu, K.-X., 2017. Impact of solid state fermentation on nutritional, physical and flavor properties of wheat bran. Food Chem. 217, 28–36. https://doi.org/10.1016/j.foodchem.2016.08.062.