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Effect of solid-state fermented and enzyme-supplemented lupins on performance and ileal amino acid digestibility in broiler chickens

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Abstract

Context. The importance of lupin in animal nutrition has increased over the years due to its moderate protein content and relative availability. Low inclusion rate in broiler diet has been associated with the presence of antinutritional factors. The established beneficial effect of fermentation as a processing strategy and dietary enzyme use in improving the nutritional value of legumes such as lupins, thus, necessitated this study.

Aims. This study determined the effect of solid-state fermented lupin (SSFLP) and enzyme-supplemented lupin (LP) on the performance and ileal amino acid digestibility in broiler chickens.

Methods. In Experiment 1 (performance trial), a total of 300 day-old Ross 308 male broiler chicks were distributed into six dietary treatments, with five replicates of 10 birds each. Diet 1 was based on a corn–soybean meal (SBM), Diets 2 and 3 contained 250 g/kg LP and 250 g/kg SSFLP respectively. Diet 4 contained 250 g/kg LP + phytase (PHY). Diet 5 contained 250 g/kg LP + xylanase (XYL). Diet 6 contained 250 g/kg LP + PHY + XYL. PHY at 500 phytase units (FTU)/kg and XYL at 1000 units/kg were added to the respective diets. In Experiment 2 (digestibility trial), a total of 240 21-day-old Ross 308 male broiler chicks was assigned to six dietary treatments with five replicate cages of eight birds each. Diet 1 was based on SBM, Diets 2 and 3 contained 650 g/kg LP and 650 g/kg SSFLP respectively. Diet 4 contained 650 g/kg LP + PHY. Diet 5 contained 650 g/kg LP + XYL. Diet 6 contained 650 g/kg LP + PHY + XYL.

Key results. Dietary inclusion of SBM and LP + PHY enhanced broiler performance from 1 to 21 days. PHY-supplemented LP diet improved birds' bodyweight gain and feed intake compared with the rest of the LP diets. Dietary inclusion of LP + PHY + XYL improved the apparent ileal digestibility (AID) of some amino acids (AA). SSF of LP had no effect on the AA contents, lowered AA digestibility and depressed weight gain in the birds. Although SSFLP diet did not improve performance, it effectively increased phosphorus (P) retention in broiler chickens. Feeding SSFLP and enzyme-supplemented LP diets enhanced the AID of calcium and P, as well as calcium retention.

Conclusions. Adding supplemental PHY to LP diet enhanced broiler performance, as indicated by the BWG and FI of these birds, which were significantly higher than those of birds fed on other LP-based diets and comparable with those of birds fed on the SBM diet from 1 to 21 days. Although the SSFLP diet did not improve broiler performance, it was effective in increasing P retention when compared with LP diets. The inclusion of LP + PHY + XYL diet improved the AID of amino acids.

Implications. SSFLP is a promising feed ingredient and can have a potential application in feed formulation. However, further studies are still needed to be able to clearly understand its effect at a high inclusion level on the performance and ileal amino acid digestibility in broiler chickens. The development of cocktail inoculants to target all ANFs in lupins will definitely open a new window for the poultry feed industry.

Keywords: digestibility, lupins, nutritive evaluation, poultry nutrition, proteins.

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Introduction

Soybean meal (SBM) is the most conventionally used plant protein source in poultry diets. Over-dependence on SBM in poultry feed has posed some economic problems associated with a reduced supply of the commodity, increase in price and competition for food and feed purposes (Zaworska *et al*. 2017; Olukomaiya *et al*. 2019*c*, 2020*a*). Thus, there is a need to explore alternative sources of protein for poultry feed. In recent times, interest in lupin (LP) as a cost-effective feed ingredient for poultry diet has increased due to its moderate protein content. However, the presence of anti-nutritional factors (ANFs), such as non-starch polysaccharides, fibre, phytic acid and the carbohydrate structure of LP, has been reported to have adverse effects on performance, thereby, limiting a high inclusion rate of LP in non-ruminant diets (Jezierny *et al*. 2010; Kim *et al*. 2011; Kasprowicz-Potocka *et al*. 2015; Zaworska *et al*. 2017). Thus, overcoming the ANFs of LP and improving the utilisation of nutrients in LP has attracted researchers' attention.

Different strategies and processing methods have been attempted to improve the nutritional value of LP for poultry diets, such as autoclaving, dehulling (Olkowski *et al*. 2001; Lee *et al*. 2016; Mera-Zúñiga *et al*. 2019), use of commercial feed enzymes (Lee et al. 2016; Mera-Zúñiga *et al*. 2019) and fermentation (Zaworska *et al*. 2017). Exogenous enzymes have been routinely used in poultry diets. Walters *et al.* (2019) and Al-Harthi *et al*. (2020) previously fed phytase at a conventional level of 500 phytase units (FTU)/kg, and the degrading of phytates by phytase supports the release of phosphorus (P), minerals, proteins, amino acids and starch (Chaves *et al*. 2020). Dietary inclusion of phytase improved feed consumption, bodyweight and feed conversion ratio, reduced mortality and enhanced protein efficiency of broiler chickens (Kong and Adeola 2011; Walters *et al*. 2019). Ghayour-Najafabadi *et al*. (2018) also fed xylanase at a conventional level of 1000 units (U)/kg. Exogenous xylanase helps to degrade non-starch polysaccharides in feed ingredients, thereby improving nutrient digestibility and the performance of broiler chickens (Selle *et al.* 2009). Most of the strategies can reduce ANFs, but SSF may be more promising in effectively reducing ANFs, improving nutritional value, minimising protein loss, reducing environmental pollution, and cheaper to use (Vig and Walia 2001; Shi *et al*. 2015).

Solid-state fermentation (SSF) has a long history of use in the food and fermentation industries (Soccol *et al*. 2017; Olukomaiya *et al*. 2019*a*, 2019*b*, 2019*c*, 2019*d*; Olukomaiya *et al*. 2020*a*, 2020*b*, 2020*c*). The beneficial effects of SSF in improving the nutritional quality of food and feed products have been previously documented (Soccol *et al*. 2017; Olukomaiya *et al*. 2019*a*, 2019*b*, 2021). In the study of Kasprowicz-Potocka *et al*. (2015), fermentation of LP seeds with *Candida utilis* increased the crude protein (CP) content and reduced phytate and oligosaccharide content. Zaworska *et al*. (2016) also found that fermentation of LP with *Saccharomyces cerevisiae* and commercial multi-bacterial preparation (*Enterococcus faecium*, *Lactobacillus plantarum*, *Lactobacillus buchneri* and *Lactobacillus casei*) induced small changes in CP, ash, fat, total alkaloids and phytate P concentrations; in contrast, it removed oligosaccharides, remarkably reduced true protein and increased crude fibre, neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents. In a later study, Zaworska *et al*. (2017) demonstrated that LP fermentation improved the contents of CP, fibre, fat, ash and most of the analysed amino acids and decreased the raffinose family oligosaccharides and phytic acid contents.

Furthermore, Kasprowicz-Potocka *et al*. (2015) found that feeding fermented LPs to rats positively affected protein digestibility of the feed, body mass gain and protein efficiency of the rats. Zaworska *et al*. (2016) also fed Wistar rats with fermented LPs and observed that the apparent total tract digestibility of dietary protein was higher in rats fed fermented LPs than in those fed raw LP diet. Zaworska *et al*. (2017) studied the influence of fermented LP on ileal digestibility and microbiota in growing pigs. The authors stated that feeding fermented LPs had a positive impact on the apparent ileal digestibility (AID) of CP and amino acids such as methionine, cysteine, isoleucine, leucine, phenylalanine and valine. To the best of our knowledge, no study has previously investigated the effect of SSFLP on performance and ileal amino acid digestibility in broiler chickens. Therefore, the objective of the present study was to determine the effect of solid-state fermented and enzyme-supplemented LPs on performance and ileal amino acid digestibility in broiler chickens.

Materials and methods

All experimental procedures used in this study were approved by the Animal Ethics Committee of the University of Queensland (AEC Approval number: SAFS/274/18). Health and animal husbandry practices complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes issued by the Australian Bureau of Animal Health (National Health and Medical Research Council 1990).

Preparation of SSFLP and enzyme sources

Lyophilised culture of *Aspergillus ficuum* (ATCC 66876) was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cracked LPs (*Lupinus angustifolius* L.) were purchased from a commercial feed mill (Allora Grain and Milling (AGM), Queensland, Australia). Solid-state fermentation of LP was conducted according to a previously described method (Olukomaiya *et al*. 2020*a*). Moisture content of the substrate was optimised at 45% using reverse osmosis water and substrate was sterilised by autoclaving at 121°C for 15 min. After cooling to room temperature, the substrate was inoculated with spore suspension of *A. ficuum* (10^7 spores/mL). Then, the mixture was incubated at 30°C for 7 days. The SSFLP was oven-dried for 48 h, cooled, ground to pass through a 1.5 mm sieve and stored at 4°C before dietary inclusion. LP and SSFLP samples were analysed for dry matter, CP, crude fat, crude ash and starch according to standard methods (AOAC 2000). Soluble carbohydrate (glucose) was measured by enzymatic method (Karkalas 1985). Most of the ingredients (in ground form) were purchased from local commercial suppliers in

Queensland, Australia. The enzymes used were phytase (AXTRA® PHY TPT 10000; (thermo-stable powder) sourced from *Buttiauxella* spp. and expressed in *Trichoderma reesei*; Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, UK) and xylanase (AXTRA® XB 201 TPT; a combination of xylanase (with enzyme activity: 12 200 U/g) and betaglucanase expressed in *Trichoderma reesei* (with enzyme activity: 1520 U/g); Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, UK) supplied by Feedworks Australia.

Experiment 1: performance trial

In total, 300 day-old Ross 308 male broiler chicks with initial average bodyweight of 38.3 ± 0.07 g were obtained from a commercial hatchery (Goulburn, New South Wales, Australia). The experiment had six dietary treatments with five replicate pens per treatment (10 birds per replicate pen) in a completely randomised design. The six experimental diets included the following: Diet 1 was based on corn–SBM, as a reference, and Diets 2 and 3 contained 250 g/kg LP and 250 g/kg SSFLP respectively. Diet 4 contained 250 g/kg LP supplemented with 500 FTU/kg phytase (PHY). Diet 5 contained 250 g/kg LP supplemented with 1000 U/kg xylanase (XYL). Diet 6 contained 250 g/kg LP supplemented with a combination of 500 FTU/kg PHY and 1000 U/kg XYL. The ingredients and nutrient composition of experimental diets are shown in Table 1. Feed and clean water were provided *ad libitum* throughout the trial. The bedding material used for pen floors was fresh wood shavings. The initial temperature was maintained at 32°C and then gradually decreased by 3°C every week. Lighting was provided for 24 h from 1 to 21 days. The trial was conducted for 21 days. At 7, 14 and 21 days, birds and feed were weighed to measure the performance parameters such as bodyweight gain (BWG), feed

Table 1. Ingredient composition of experimental diets (g/kg as-fed basis; Experiment 1)
SBM, soybean meal; LP, lupin; SSFLP, solid-state fermented lupin

Ingredient	Diet					
	SBM	LP	SSFLP	LP + PHY	LP + XYL	LP + PHY + XYL
Corn	550	395	395	395	395	395
Soybean meal	359	231	231	231	231	231
Lupin	–	250	–	250	250	250
SSFLP	–	–	250	–	–	–
Meat and bone meal	50	50	50	50	50	50
Canola oil	20	51	51	51	51	51
Lysine	1.5	1.4	1.4	1.4	1.4	1.4
DL-Methionine	2.7	3.5	3.5	3.5	3.5	3.5
L-Threonine	0.6	1.1	1.1	1.1	1.1	1.1
Arginine	–	–	–	–	–	–
Isoleucine	–	0.8	0.8	0.8	0.8	0.8
Valine	–	0.3	0.3	0.3	0.3	0.3
Limestone	2.4	2.5	2.5	2.5	2.5	2.5
Monodicalcium phosphate	4.0	4.1	4.1	4.1	4.1	4.1
Sodium chloride	2.1	2.3	2.3	2.3	2.3	2.3
Sodium bicarbonate	2.0	1.3	1.3	1.3	1.3	1.3
Vitamin and mineral premix[A]	5	5	5	5	5	5
Choline chloride	0.7	0.7	0.7	0.7	0.7	0.7
PHY[B]	–	–	–	+	–	+
XYL[C]	–	–	–	–	+	+
	Nutrient composition (g/kg)					
ME (kcal/kg)	3026	3025	3025	3025	3025	3025
Crude protein	244	248	248	248	248	248
Lysine	14.3	14.6	14.6	14.6	14.6	14.6
Methionine	6.6	6.8	6.8	6.8	6.8	6.8
Met + Cys	10.2	10.4	10.4	10.4	10.4	10.4
Cystine	3.6	3.6	3.6	3.6	3.6	3.6
Calcium	8.1	8.1	8.1	8.1	8.1	8.1
Available phosphorus	4.5	4.5	4.5	4.5	4.5	4.5
Sodium	1.8	1.8	1.8	1.8	1.8	1.8
Chloride	2.3	2.5	2.5	2.5	2.5	2.5

[A]Each 2 kg contains the following: vitamin A, 10 milli international units (MIU); vitamin D3, 2.5 MIU; vitamin E, 30 g; vitamin K3, 2 g; vitamin B1, 1.5 g; vitamin B2, 8 g; vitamin B6, 4 g; vitamin B12, 20 mg; vitamin B5, 14 g; vitamin B9, 2 g; vitamin B3, 45 g; vitamin H, 135 mg; cobalt, 20 mg; copper, 6 g; iron, 50 g; iodine, 750 mg; manganese, 75 g; molybdenum, 1 g; selenium, 150 mg; zinc, 60 g.
[B]PHY: phytase added at 500 FTU/kg.
[C]XYL: xylanase added at 1000 U/kg.

intake (FI) and feed conversion ratio (FCR). Mortality in each replicate pen was recorded daily as it occurred, and corrections on BWG, FI and FCR were made accordingly.

Experiment 2: apparent metabolisable energy and amino acid digestibility assay

In total, 240 21-day-old Ross 308 male broiler chicks were used for the digestibility study. The experiment had six dietary treatments with five replicate cages per treatment (eight birds per replicate cage) in a completely randomised design. Dietary protein in the assay diets was supplied solely by the test ingredients (Bryden *et al*. 2009; Olukomaiya *et al*. 2021). The chicks were arranged in cages (90 × 75 × 45 cm), which had a linear feeder in the front and a nipple drinker at the back, in an environmentally controlled room. The six experimental diets included the following: Diet 1 was based on SBM, as a reference; Diets 2 and 3 contained 650 g/kg LP and 650 g/kg SSFLP respectively; Diet 4 contained 650 g/kg LP supplemented with 500 FTU/kg PHY; Diet 5 contained 650 g/kg LP supplemented with 1000 U/kg XYL; and Diet 6 contained 650 g/kg LP supplemented with a combination of 500 FTU/kg PHY and 1000 U/kg XYL. The composition of the diets used in the amino acid digestibility assay is presented in Table 2. The assay diets were offered *ad libitum* for 4 days, and water was available at all times. Celite® (Celite Corporation, Lompoc, CA, USA), a source of acid-insoluble ash (AIA) was added to all diets at 20 g/kg as an indigestible marker. The representative excreta samples were collected in the last two consecutive days, and care was taken to avoid contamination with feathers, scales and debris. The excreta sample from each replicate cage was dried at 85°C for 48 h. The dried excreta samples were later ground to pass through a 0.5 mm sieve and stored in airtight containers at –20°C for the analysis of AIA, gross energy (GE), calcium (Ca) and P. On Day 25, all birds were killed by cervical dislocation (Mera-Zúñiga *et al*. 2019) and the contents of the lower half of the ileum were collected. The ileum was defined as the portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileo–caecal junction. The ileum was divided into two halves, and the digesta were collected from the lower half towards the ileo–caecal junction. The pooled ileal digesta contents from each cage were immediately frozen at –20°C and then freeze-dried. Samples of the assay diets and freeze-dried ileal digesta samples were then finely ground to pass through a 0.5 mm sieve and stored in airtight containers at –20°C for the analysis of AIA, CP, Ca, P and amino acid contents.

Chemical analysis

The GE contents of diet and excreta samples were determined from a 1 g sample using an adiabatic bomb calorimeter (IKA® – WERKE, C2000, GMBH and Co., KG, Staufen, Germany), which had been standardised with benzoic acid. Amino acid composition of the diets and ileal digesta samples was determined at the Department of Molecular Science, Australian Proteome Analysis Facility (Macquarie University, New South Wales, Australia). Amino acid profile analysis was performed as per Australian Proteome Analysis Facility standard operating procedure (AAA-001). Samples (100 mg per replicate) were first hydrolysed with 6 M hydrochloric acid at 110°C for 24 h. Under these conditions, asparagine was hydrolysed to aspartic acid and glutamine to glutamic acid; thus, the reported amount of these acids is the sum of those respective components. After hydrolysis, all amino acids were labelled using the Waters AccQTag Ultra chemistry (following supplier's recommendations) and analysed on a Waters Acquity UPLC. Cystine and tryptophan concentrations were not determined. Nitrogen

Table 2. Ingredient composition of experimental diets used in the amino acid digestibility assay (Experiment 2; g/kg as-fed basis)

SBM, soybean meal; LP, lupin; SSFLP, solid-state fermented lupin

Ingredient	Diet					
	SBM	LP	SSFLP	LP + PHY	LP + XYL	LP + PHY + XYL
Dextrose	505	244	244	244	244	244
Test ingredient	402	650	650	650	650	650
Canola oil	60	60	60	60	60	60
Limestone	3	4	4	4	4	4
Sodium chloride	2	2	2	2	2	2
Sodium bicarbonate	–	2	2	2	2	2
Vitamin and mineral premix[A]	5	5	5	5	5	5
Choline chloride	3	3	3	3	3	3
Celite	20	20	20	20	20	20
Cellulose	–	10	10	10	10	10
PHY[B]	–	–	–	+	–	+
XYL[C]	–	–	–	–	+	+

[A]Each 2 kg contains the following: vitamin A, 10 milli international units (MIU); vitamin D3, 2.5 MIU; vitamin E, 30 g; vitamin K3, 2 g; vitamin B1, 1.5 g; vitamin B2, 8 g; vitamin B6, 4 g; vitamin B12, 20 mg; vitamin B5, 14 g; vitamin B9, 2 g; vitamin B3, 45 g; vitamin H, 135 mg; cobalt, 20 mg; copper, 6 g; iron, 50 g; iodine, 750 mg; manganese, 75 g; molybdenum, 1 g; selenium, 150 mg; zinc, 60 g.

[B]PHY: phytase added at 500 FTU/kg.

[C]XYL: xylanase added at 1000 U/kg.

(N) concentration was determined on a 0.25 g sample with a N analyser (Elementar vario MACRO CHN/CHNS, Hanau, Germany). The CP content of samples was calculated by multiplying percentage N by a correction factor of 6.25. The AIA contents of diets, ileal digesta and excreta samples were measured after ashing the samples and treating the ash with boiling 4 mol/L hydrochloric acid (Mollah *et al*. 1983).

Digestibility and apparent metabolisable energy (AME) calculation

Apparent ileal digestibility of CP and amino acids was calculated using AIA as an indigestible marker. Celite was added to assay diets to increase the AIA fraction and to improve the precision of the measurement.

The AID and AME were determined by the marker method, by using the following equations, as performed by Robbins and Firman (2006) on ileal digesta:

$$\text{AID}\,(\%) = [1-[(\text{AIA in diet}/\text{AIA in ileal digesta}) \times (\text{Nutrient concentration in ileal digesta}/\text{Nutrient concentration in diet})]] \times 100$$

$$\text{AME}\,(\text{MJ/kg}) = \text{GE in diet} \times [1 - (\text{AIA in diet}/\text{AIA in excreta}) \times (\text{GE in excreta}/\text{GE in diet})]$$

$$\text{Nutrient retention}\,(\%) = [\text{Nutrient in diet}-[\text{Nutrient in excreta} \times (\text{marker in diet}/\text{marker in excreta})]] \times 100,$$

where AID% = percentage AID; AIA = acid insoluble ash; AME = apparent metabolisable energy; GE = gross energy.

Statistical analyses

The data collected were analysed by one-way ANOVA using the IBM SPSS Statistics V.25 (IBM Corp., NY, USA). Differences were considered to be significant when $P < 0.05$. Significant differences between means were separated using the Tukey's procedure.

Results

The analysed chemical composition of LP and SSFLP used in the present study was presented in an earlier report (Olukomaiya *et al*. 2020*a*). The LP used was analysed to contain 358 g/kg CP, 61 g/kg ether extract, 36 g/kg crude ash, 1 g/kg Ca, 3 g/kg P, 289 g/kg NDF and 208 g/kg ADF. Also, SSFLP used contained 353 g/kg CP, 63 g/kg ether extract, 37 g/kg crude ash, 1 g/kg Ca, 4 g/kg P, 374 g/kg NDF and 230 g/kg ADF. The fibre fractions were similar in the LP and SSFLP. The analysed amino acid composition of SBM, LP and SSFLP is presented in Table 3. Arginine was the most abundant essential amino acid, while glutamic acid was found to be the most abundant non-essential amino acid.

Table 3. Analysed crude protein and amino acid composition of ingredients (g/kg DM basis)

Item	Ingredient		
	Soybean meal	Lupin	Solid-state fermented lupin
Crude protein	475	358	353
Essential amino acids[A]			
Arginine	38.1	33.3	29.5
Histidine	14.1	8.9	8.0
Isoleucine	25.6	14.0	13.8
Leucine	42.7	22.8	22.8
Lysine	31.9	15.9	11.8
Methionine	6.1	1.9	1.6
Phenylalanine	27.9	13.3	12.8
Threonine	21.7	11.8	11.5
Valine	26.4	13.5	13.3
Non-essential amino acids[A]			
Alanine	22.4	11.0	11.0
Aspartic acid	57.2	33.3	31.8
Glutamic acid	94.9	69.9	67.4
Glycine	23.0	14.1	14.0
Proline	27.5	13.5	13.4
Serine	27.9	15.9	15.6
Tyrosine	15.6	9.2	8.5

[A]Analyses were performed in duplicate.

Performance of broiler chickens

There was only one mortality in total (data not shown), and birds looked healthy throughout the trial. Effect of experimental diets on performance of broiler chickens from 1 to 21 days post-hatch is presented in Table 4. From 1 to 7 days, dietary treatments did not have any effect ($P > 0.05$) on the performance of the broiler chickens. From 8 to 14 days, broiler chickens fed SBM and LP + PHY diets had a significantly ($P < 0.05$) higher BWG than those in other treatments. Broiler chickens fed SBM and LP + PHY diets showed a significantly ($P < 0.05$) higher FI than did those fed SSFLP, LP + XYL and LP + PHY + XYL diets, but did not differ significantly ($P > 0.05$) from those fed the LP diet. The dietary treatments did not have any effect ($P > 0.05$) on the FCR of the birds. From 15 to 21 days, broiler chickens fed SSFLP and LP diets showed lower ($P < 0.05$) BWG than birds fed the SBM diet. The SSFLP diet produced the lowest ($P < 0.05$) BWG among all treatments. The BWG in birds fed the SBM diets did not differ significantly ($P > 0.05$) from that of birds fed enzyme-supplemented LP diets. Broiler chickens fed the SBM diet had the highest ($P < 0.05$) FI, which was not significantly ($P > 0.05$) different from those fed the LP + PHY diet. Feeding the SSFLP diet resulted in the lowest ($P < 0.05$) FI compared with other treatments. The FCR was improved ($P < 0.05$) in broiler chickens fed SBM and LP-based diets, except in those fed the SSFLP diet. During the entire experimental period (1–21 days), broiler chickens fed SBM and LP + PHY diets had the highest BWG and FI, and these were significantly ($P < 0.05$) higher than those of other treatments. It was also observed that the dietary inclusion of SBM and the enzyme-supplemented LP diets significantly ($P < 0.05$) improved the FCR in the birds when compared with the SSFLP diet.

AME and amino acid digestibility

The effect of experimental diets on AID of nutrients and amino acids in broiler chickens at 25 days of age is presented in Table 5. The AME of the SBM diet was the highest ($P < 0.05$)

Table 4. Effect of experimental diets on the performance of broiler chickens from 1 to 21 days post-hatch (Experiment 1)

Values are the mean of five replicates. BWG, bodyweight gain; FI, feed intake; FCR, feed conversion ratio; SBM, soybean meal; LP, lupin; SSFLP, solid-state fermented lupin; LP + PHY, lupin + phytase; LP + XYL, lupin + xylanase; LP + PHY + XYL, lupin + phytase + xylanase; PHY, phytase added at 500 FTU/kg; XYL, xylanase added at 1000 U/kg; s.e.m., standard error of the mean. Means in the same column followed by different lowercase letters are significantly different (at $P = 0.05$)

Diet	1–7 days			8–14 days			15–21 days			1–21 days		
	BWG (g/bird)	FI (g/bird)	FCR (g/g)	BWG (g/bird)	FI (g/bird)	FCR (g/g)	BWG (g/bird)	FI (g/bird)	FCR (g/g)	BWG (g/bird)	FI (g/bird)	FCR (g/g)
SBM	127	128	1.01	324a	376a	1.16	584a	694a	1.19b	1035a	1198a	1.16b
LP	121	121	1.00	292b	356ab	1.22	534b	641bc	1.20b	947b	1118b	1.18ab
SSFLP	123	123	1.00	289b	350b	1.21	482c	619c	1.28a	895c	1092b	1.22a
LP + PHY	128	124	0.97	318a	372a	1.17	577ab	683ab	1.18b	1022a	1180a	1.15b
LP + XYL	122	119	0.98	295b	343b	1.16	543ab	649bc	1.20b	960b	1112b	1.16b
LP + PHY + XYL	120	122	1.02	289b	350b	1.21	561ab	654abc	1.17b	970b	1126b	1.16b
s.e.m.	1.05	1.13	0.01	3.92	2.90	0.01	7.52	5.98	0.01	10.41	8.56	0.01

Table 5. Effect of experimental diets on apparent ileal digestibility (%) of nutrients and amino acids in broiler chickens at 25 days of age (Experiment 2)

AME values relate to the diet and digestibility to ingredients. Each value represents the mean of five replicates. AME, apparent metabolisable energy; CP, crude protein; SBM, soybean meal; LP, lupin; SSFLP, solid-state fermented lupin; PHY, phytase added at 500 FTU/kg; XYL, xylanase added at 1000 U/kg; s.e.m., standard error of the mean. Means in the same row followed by different lowercase letters are significantly different (at $P = 0.05$)

Item	Diet						
	SBM	LP	SSFLP	LP + PHY	LP + XYL	LP + PHY+ XYL	s.e.m.
AME (MJ/kg)	15.0a	11.0b	12.0b	11.0b	11.0b	10.0b	0.35
CP	88.0	85.0	81.0	82.0	86.0	89.0	1.12
Essential amino acids							
Arginine	92.8bc	94.7ab	92.3c	93.1abc	93.1abc	95.1a	0.27
Histidine	89.1	87.5	82.4	85.0	84.5	88.0	0.77
Isoleucine	88.5	86.1	86.9	85.1	84.3	88.3	0.49
Leucine	88.8	87.4	88.6	87.0	86.0	89.6	0.42
Lysine	89.0a	85.5a	80.3b	87.2a	86.4a	88.7a	0.66
Methionine	92.4a	85.3b	77.8c	86.0b	79.3c	89.2ab	1.07
Phenylalanine	89.8	87.9	88.9	87.3	86.5	89.7	0.41
Threonine	80.4	79.1	80.4	77.0	78.5	83.0	0.69
Valine	86.7	83.4	85.9	82.6	82.9	86.6	0.56
Mean	88.6	86.3	84.8	85.6	84.6	88.7	0.52
Non-essential amino acids							
Alanine	85.0	79.7	85.0	82.4	81.2	84.7	0.64
Aspartic acid	82.6b	85.1ab	81.5b	85.2ab	85.4ab	88.3a	0.60
Glutamic acid	88.7b	90.2ab	90.4ab	90.9ab	90.0ab	92.3a	0.35
Glycine	82.3	82.7	81.9	83.5	82.3	86.0	0.55
Proline	85.5	81.7	84.1	81.7	81.3	85.3	0.61
Serine	85.6	83.2	82.3	81.9	82.1	86.1	0.56
Tyrosine	90.0	89.8	88.0	87.9	87.1	90.9	0.41
Mean	85.7	84.6	84.7	84.8	84.2	87.7	0.36

among all treatments, while the LP-based diets showed similar ($P > 0.05$) AME values. There was no significant ($P > 0.05$) difference in AID of CP among the experimental diets. The AID of amino acids was similar across the dietary treatments except for arginine, lysine, methionine, aspartic acid and glutamic acid. The LP + PHY + XYL diet was characterised by the highest ($P < 0.05$) AID for arginine, which was similar to that of the LP diet, while the lowest value was found in SSFLP diets. Compared with other treatments, SSFLP diet was observed to have the lowest ($P < 0.05$) AID for lysine. The AID of methionine in the SBM diet was highest ($P < 0.05$) among the treatments but statistically similar ($P > 0.05$) to that of the LP + PHY + XYL diet. The SSFLP and LP + XYL diets recorded the lowest ($P < 0.05$) AID for methionine. The AID for aspartic acid in LP + PHY + XYL diet was observed to be higher ($P < 0.05$)

Table 6. Effect of experimental diets on apparent ileal digestibility and retention (%) of calcium and phosphorus in broiler chickens at 25 days of age (Experiment 2)

Values are the mean of five replicates. SBM, soybean meal; LP, lupin; SSFLP, solid-state fermented lupin; LP + PHY, lupin + phytase; LP + XYL, lupin+ xylanase; LP + PHY + XYL, lupin+ phytase + xylanase; PHY, phytase added at 500 FTU/kg; XYL, xylanase added at 1000 U/kg; s.e.m., standard error of the mean. Means in the same row followed by different lowercase letters are significantly different (at $P = 0.05$)

Diet	Ileal digestibility (%)		Retention (%)	
	Calcium	Phosphorus	Calcium	Phosphorus
SBM	57b	64c	48b	53ab
LP	72a	69bc	66a	43abc
SSFLP	77a	75ab	72a	54a
LP + PHY	73a	73ab	62ab	31c
LP + XYL	71a	63c	67a	31c
LP+ PHY+ XYL	67ab	79a	64ab	36bc
s.e.m.	1.53	1.23	1.91	2.28

than that of SBM and SSFLP diets, while that found in LP, LP + PHY and LP + XYL diets was intermediate. The AID of glutamic acid in the LP + PHY + XYL diet was higher ($P < 0.05$) than that of the SBM diet, but did not differ ($P > 0.05$) from that of the other LP-based diets. The effect of experimental diets on AID and retention of Ca and P in broiler chickens at 25 days of age is presented in Table 6. The AID of Ca in LP, SSFLP, LP + PHY and LP + XYL diets was higher ($P < 0.05$) than that in the SBM diet, but not different ($P > 0.05$) from that of the LP + PHY + XYL diet. The AID of P in the LP + PHY + XYL diet was higher ($P < 0.05$) than that in SBM and LP+XYL diets, but similar ($P > 0.05$) to that of SSFLP and LP + PHY diets. Ca retention in LP, SSFLP and LP + XYL diets was higher ($P < 0.05$) than that in the SBM diet, but did not differ ($P > 0.05$) from that in LP + PHY and LP + PHY + XYL diets. P retention in SSFLP diet was the highest ($P < 0.05$) among the treatments, but statistically similar ($P > 0.05$) to that of the SBM diet.

Discussion

Performance of broiler chickens

The effect of inclusion of LP at 250 g/kg in broiler diets has been previously reported (Roth-Maier and Paulicks 2003; Smulikowska *et al*. 2014; Mera-Zúñiga *et al*. 2019). In the report of Steenfeldt *et al*. (2003), dietary inclusion of LP at 200 g/kg significantly reduced BWG and FCR in broilers from 7 to 21 days of age. In the present study, from 8 to 14 days, broiler chickens fed SBM and LP + PHY diets had higher BWG and FI than did those in the other treatments. A similar trend was also observed during the entire experimental period (1–21 days), with birds fed the SBM diet and the 250 g/kg LP diet supplemented with PHY showing the highest BWG and FI compared with those in the other treatments. In line with the present finding, the positive influence of PHY supplementation on broiler performance has been well established (Wu *et al*. 2004; Cowieson *et al*. 2006; Selle *et al*. 2012; Babatunde *et al*. 2019; Ciurescu *et al*. 2020; Attia *et al*. 2020). However, Woyengo *et al*. (2008) did not observe any improvement in performance as a result of supplementing broiler diet with PHY. This improvement in broiler response may be attributed to the hydrolysis of phytate complexes, leading to an increased release of proteins and amino acids for digestion and absorption (Selle *et al*. 2000; Onyango *et al*. 2004).

Unlike the trend observed with the LP + PHY diet, the LP + XYL diet and the LP + PHY + XYL diet did not enhance the performance of broilers from 1 to 21 days. Conflicting evidence exists on the impact of XYL and the combination of PHY and XYL on broiler performance. For instance, Wu *et al*. (2004) reported improvement by XYL on the performance in broiler chickens fed wheat-based diets, whereas Pekel *et al*. (2017) reported that XYL did not improve nutrient utilisation and performance of broiler chickens fed camelina meal-based diets. Furthermore, Woyengo *et al*. (2008) did not observe any improvement in performance as a result of supplementing broiler diet with PHY and XYL, while Selle *et al*. (2003) reported that the inclusion of PHY and XYL improved BWG and feed efficiency of broilers (7–28 days post-hatch). With regards to our results, the response to feed enzymes is dependent on many factors such as diet composition as well as the source and level of enzyme addition (Bryden and Li 2010).

To the best of our knowledge, this is the first study to investigate the nutritional value of SSFLP in broiler chickens. From 15 to 21 days and from 1 to 21 days, dietary inclusion of SSFLP did not result in improved broiler performance, as expected. Similarly, Siriwan *et al*. (2005*a*, 2005*b*) reported that fermented common beans included at 5–20% in poultry diet reduced FI, BWG and feed efficiency. In contrast to the present findings, Chiang *et al*. (2010) and Xu *et al*. (2012) reported a beneficial effect of feeding fermented rapeseed meal on broiler performance. The reduced performance may partly be due to the fibre content of the SSFLP diet, as was previously reported by Olukomaiya *et al*. (2020*a*). The SSFLP used in the present study had numerically higher NDF and ADF contents, although they were not significantly higher than those of the LP. The fibre content in SSFLP diet may be responsible for the reduced performance, resulting in poor BWG, FI and FCR. High fibre content adversely affects broilers at an early age, causing reduced performance (Khajali and Slominski 2012; Gopinger *et al*. 2014). Under SSF conditions, microbial activities alter the physicochemical characteristics of substrates, thereby changing many features of the resultant product (Ajila *et al*. 2012; Olukomaiya *et al*. 2019*c*). It was also observed that the dietary inclusion of SBM and enzyme-supplemented LP diets enhanced the FCR in the birds when compared with the SSFLP diet, from 1 to 21 days. The improvement observed in the FCR from 1 to 21 days may be due to a marginal increase in the BWG of the broiler chickens relative to FI.

Overall, relative to feeding SBM and LP diets, the enzyme-supplemented diets were found to induce improved performance compared with the SSFLP diets in the birds from 1 to 21 days. The reason for this observation is not clear and, thus, more studies are still needed to better understand the nutritional value of SSFLP for broiler performance.

AME and amino acid digestibility

The amino acid contents and AID values determined for SBM and LP diets in the present study were similar to those reported previously (Bryden *et al*. 2009; Nalle *et al*. 2011). The beneficial role of enzyme supplementation in improving amino acid digestibility in broilers has been well demonstrated (Selle *et al*. 2003; Bryden and Li 2004; Selle *et al*. 2009).

In the present study, the most pronounced improvements in AID of amino acids were associated with the combined supplementation of PHY and XYL in the LP diet. The positive impact confirmed the synergistic impact of PHY and XYL on the AID of arginine, aspartic acid and glutamic acid. This finding is in agreement with the report of Selle *et al*. (2003) who noted similar synergistic responses for most of the amino acids subsequent to the addition of PHY and XYL to wheat-based diets. It is possible that the combined supplementation of PHY and XYL to the LP-based diet may have a supportive manner of action in improving the AID of amino acids. The improvement in AID of amino acids by both PHY and XYL may also be due to the decrease of endogenous amino acid flows (Selle *et al*. 2009). Low AID of methionine was observed in the LP + XYL diet compared with the SBM or LP diet, although this reduction was similar to that of the SSFLP diet. Selle *et al*. (2003) also reported that XYL had no effect on the AID of amino acids in broilers fed wheat-based diets. This was an unforeseen result as the ability of XYL to enhance the AID of amino acids has been previously demonstrated (Hew *et al*. 1998). Methionine has a minimal possibility for improvement with PHY and XYL supplementation, because basal digestibility at the terminal ileum is naturally greater than 90% in a corn–SBM diet (Rutherfurd *et al*. 2004; Gehring *et al*. 2013).

In general, the enzyme-supplemented LP diets had significant effects on AID of amino acids compared with the SSFLP diet. Ahmed *et al*. (2014) reported that SSF enhanced AID of amino acids in canola meal. Zaworska *et al*. (2017) also found that fermentation had a positive impact on the AID of amino acids in LP-based diets. However, these reports do not agree with the results of the present study. With regards to the present study, it is possible that processing conditions before incorporation into the diet may have accounted for the poor AID of amino acids observed in the SSFLP diet. Processing condition is an important factor that has been found to modify amino acid digestibility (Bryden and Li 2004).

It is noteworthy that it has been shown that excess Ca and P absorbed from the intestine can be excreted from urine into excreta (Li *et al*. 2017), which may be responsible for nutrient retention values being lower than nutrient digestibility in the present study. The positive effects of PHY and XYL on Ca digestibility have been reported by many authors (Ravindran *et al*. 2008; Khoramabadi *et al*. 2014; Hosseini and Afshar 2017; Fernandes *et al*. 2019). In the present study, birds offered SSFLP and enzyme-supplemented LP diets generated an improved response in the AID of Ca. Also, AID of P was observed to improve in birds fed the LP + PHY + XYL diet relative to those fed the SBM or LP diets. Likewise, the improvement in Ca retention noted for LP, SSFLP and LP + XYL diets and P retention in SSFLP diet is of pivotal importance. In line with the present findings, Moss *et al*. (2018) reported that the combination of PHY and XYL in birds offered canola meal-based diets increased the ileal digestibility of Ca and P. The synergistic action of PHY and XYL can be beneficial through improved access to cell contents in the intestinal environment (Adeola and Cowieson 2011).

Ramesh and Chandrasekaran (2011) demonstrated that pure enzyme supplementation increased retention of Ca and P in birds. Ca is poorly digested in broiler diets, as Amerah *et al*. (2014) found that AID of Ca ranged from 54% to 61.8% in unsupplemented corn–SBM diets, which aligns with the AID of Ca in the SBM diet in the present study, which was analysed to be 57%. The increase in Ca and P digestibilities and retention may be explained by a clear and large breakdown of mineral–phytate complexes or the release of Ca and P from soluble fibre-bound Ca and P (Pekel *et al*. 2017). PHY catalyses the dephosphorisation reaction of phytic acid in inorganic phosphate and smaller esters that have low chelate capacity and make nutrients unavailable (Lei and Porres 2003).

In our study, reduced P digestibility in the LP + XYL diet and P retention in the LP + PHY and LP + XYL diets were observed. Similarly, XYL supplementation did not have an effect on ileal nutrient digestibility (Pekel *et al*. 2017). It is not clear which factors triggered the difference in response to PHY or XYL supplementation on ileal P digestibility and retention in the current study. With regards to the SSFLP diet, Chiang *et al*. (2010) and Mandey *et al*. (2015) also reported that Ca digestibility and retention were increased in fermented feeds compared with the unfermented control. The improvement in Ca and P digestibility and retention in SSFLP diet may be attributed to the chelating effect of organic acids from SSF on Ca (Centeno *et al*. 2007). During fermentation, microbial enzymes can hydrolyse the phytate complex (Ahmad *et al*. 2000; Sugiharto and Ranjitkar 2019), with increased phytate solubility and susceptibility to hydrolysis (Centeno *et al*. 2007; Esmaeilipour *et al*. 2011), thereby increasing the retention and utilisation of Ca and P.

Conclusions

In conclusion, supplementing LP diet with PHY enhanced the performance of broiler chickens, as indicated by the BWG and FI of these birds, which were significantly higher than those of the birds fed on other LP-based diets and comparable to those birds fed on the SBM diet, from 1 to 21 days. Although the SSFLP diet did not improve broiler performance, it was effective in increasing P retention in broiler chickens. The inclusion of LP + PHY + XYL in the diet improved the AID of amino acids. Feeding SSFLP and enzyme-supplemented LP diets enhanced Ca and P digestibility as well as Ca retention. SSFLP is a promising feed ingredient and can have a potential application in feed formulation. However, further studies are still needed to be able to clearly understand its practical application at a high inclusion level on performance and ileal amino acid digestibility in broiler chickens. The

development of cocktail inoculants to target all ANFs in LPs will definitely open a new window for the poultry feed industry.

Conflicts of interest

The authors declare no conflicts of interest.

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