Influence of microbial fermentation processing of sesame meal and enzyme supplementation on broiler performances

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ABSTRACT

Two experiments were conducted to investigate the effects of fermented sesame meal (FSM) replacement by soybean meal (SBM) in broilers. In the first experiment, a completely randomised design with $3 \times 4$ factorial arrangement was used for microorganism comparison [Lactobacillus acidophilus (LA), Saccharomyces cerevisiae (SC) and LA + SC] and fermentation time courses (0, 2, 7, and 12 days). In the second experiment, a total of 420-day-old boilers (Ross 308V) were randomly allocated to seven treatments and five replicates. The experimental treatments include basal diet based on soybean meal (SBM), SBM substituted with 15 and 25% raw sesame meal (RSM) with and without phytase (PHX); RSM$_{15}$, RSM$_{25}$, RSM$_{15}$ + PHX, and RSM$_{25}$ + PHX, and SBM substituted with 15 and 25% FSM; FSM$_{15}$ and FSM$_{25}$. Results showed that, in the fermentation process, the main effects of microorganism and days of fermentation significantly affected the pH and crude protein ($p < .05$). Also, the main effect of day of fermentation was found significant for phytic acid, oxalate and crude fibre ($p < .05$). The crude protein was increased when a mixture of LA and SC was used ($p < .05$). Broilers fed RSM$_{25}$ had the lowest and the highest BWG and FCR, respectively, than those fed other diets on day 42 ($p < .05$). Compared to all diets except for RSM$_{15}$, RSM$_{25}$ a reduction was recorded for phosphorus digestibility ($p < .05$). In conclusion, fermentation process improved the nutrient value of the RSM and subsequently performance of the broilers and could be used as a protein source in broiler diets.

HIGHLIGHTS

- Fermentation process leads to significant decrease in phytic acid content of sesame meal.
- Raw sesame meal had adverse effects on the performance of broilers.
- Fermented sesame meal may improve the broilers performance similar to soybean meal.

Introduction

Raw sesame meal (RSM) is the residue after oil extracting from sesame seed. There are many scientific reports on the nutritional values of sesame meal. The protein content of RSM is up to 40–60% (Pan et al. 1992; Mamputu and Buhr 1995); therefore, it may be considered as a suitable alternative for soybean meal. The RSM is also a rich source of minerals; though, its mineral availability is lower due to high levels of oxalate and phytate. In addition, Ravindran and Blair (1992) proposed that substitution of RSM in broiler’s starter diets is limited because of its high fibre content and possible availability problems associated with phytate and oxalate. Meanwhile, results from different studies revealed that, substitution of soybean meal with RSM could be safe up to 15%. Various processing methods of RSM, such as heating, soaking, phytase enzyme supplementation, and fermentation have been found with notable effects on its nutrient quality and accessibility; hence, the nutrient composition of RSM varies widely depending on the processing methods. For instance, Lease and Williams (1967) showed that high level of oxalate and phytic acid of RSM could be reduced under heating process. The usefulness of phytase to improve phosphorous and Calcium digestibility in poultry nutrition is generally accepted and substantial number of scientific reports provide clear evidence for these effects (Lei and Stahl 2001; Ravindran et al. 2006; Selle and Ravindran 2007). Also, a significant
relationship was reported between the proportion of dietary phytic acid destruction by exogenous phytase and the ileal amino acid digestibility coefficients, where phytase effects ranged from 2–3% to 9–10% as phytic acid destruction increased from <10% to around 50% (Cowieson et al. 2017). Another research finding showed that soaking of whole and dehulled black sesame seeds in water decreased oxalate contents by 26.78% and 29.64%, respectively (Makinde and Akinoso 2013). Regarding the fermentation, it has been reported that anti-nutritional factors (e.g. tannin, phytic acid and trypsin inhibitor) of fermented sesame oil cake by *Bacillus subtilis* subsp were lower than that of unfermented sesame oil cake (Das and Ghosh 2015). Olude et al. (2016) demonstrated that reduction in phytic acid and tannin of RSM as a result of lactic acid fermentation. Furthermore, it has been shown that the fermented sesame meal (FSM) by *Lactobacillus acidophilus* (LA) completely removed its phytic acid content (Mukhopadhyay and Ray 1999b, 1999a). Also, Hassaan et al. (2015) reported that submerged fermentation of soybean meal with *Saccharomyces cerevisiae* (SC) reduced its phytic acid content lower than detectable levels, which may provide nutritional benefits to monogastric animals, particularly weaned pigs. To the best of our knowledge, there is no study to investigate the effect of FSM on the productivity of broiler chickens. Based on such findings, the assumption is that fermentation of RSM by *L. acidophilus* and *S. cerevisiae* could promising increase nutrient utilisation and subsequently cause an improvement in growth performance of broiler chickens. Results from different studies revealed that the *L. acidophilus* (Onipede et al. 2020) and *S. cerevisiae* (Foster and Nakata 2014; Olukomaiya et al. 2020) have an enzymatic potential to improve nutrient utilisation and accessibility of nutrients of legume seeds in the livestock diets by decreasing anti-nutritional factors.

### Materials and methods

Two experiments were designed. In the first experiment, the individual and combinatorial effects of LA and SC on the nutrient availability of RSM in different time (0, 2, 7 and 12 days) were investigated. Based on the findings of this step, the duration and the microbial combination of fermentation were determined. In the second experiment, the effects of sesame meal processed by fermentation and phytase enzyme on the performance, nutrient digestibility, digestive enzyme activities, carcass characteristics, digestive tracts development and lymphoid organs in broiler chickens were investigated.

| Microorganism | Day | pH | Dry matter, % | Crude protein, % | Ether extract, % | ASH, % | Crude fibre, % | Phytic acid, % | Oxalate, % |
|---------------|-----|----|---------------|-----------------|-----------------|-------|---------------|---------------|------------|
| LA            | 0   | 6.03 | 95.41        | 45.85           | 13.95           | 10.81 | 3.51          | 1.33          | 0.609      |
|               | 2   | 4.46 | 94.74        | 46.20           | 14.52           | 9.67  | 2.34          | 1.28          | 0.495      |
|               | 7   | 4.29 | 94.84        | 47.62           | 14.78           | 9.50  | 2.36          | 1.01          | 0.374      |
|               | 12  | 4.27 | 94.25        | 47.56           | 15.17           | 9.42  | 2.39          | 0.81          | 0.327      |
| SC            | 0   | 5.98 | 94.84        | 46.01           | 14.01           | 10.72 | 3.48          | 1.31          | 0.610      |
|               | 2   | 5.50 | 94.59        | 45.99           | 15.72           | 9.98  | 2.92          | 1.10          | 0.440      |
|               | 7   | 5.40 | 93.76        | 48.81           | 16.19           | 9.74  | 2.80          | 1.11          | 0.396      |
|               | 12  | 4.46 | 94.26        | 49.16           | 16.11           | 9.54  | 2.46          | 0.80          | 0.354      |
| LA + SC       | 0   | 6.04 | 94.40        | 46.14           | 14.05           | 10.66 | 3.50          | 1.32          | 0.607      |
|               | 2   | 4.96 | 92.60        | 50.38           | 15.19           | 10.04 | 2.90          | 1.22          | 0.466      |
|               | 7   | 4.52 | 94.22        | 51.37           | 15.62           | 9.66  | 2.35          | 0.81          | 0.323      |
|               | 12  | 4.19 | 93.26        | 52.84           | 15.85           | 9.55  | 2.09          | 0.74          | 0.296      |

**Main effects**

| Microorganism | Day | pH | Dry matter, % | Crude protein, % | Ether extract, % | ASH, % | Crude fibre, % | Phytic acid, % | Oxalate, % |
|---------------|-----|----|---------------|-----------------|-----------------|-------|---------------|---------------|------------|
| LA            | 0   | 6.01 | 95.41        | 45.85           | 13.95           | 10.81 | 3.51          | 1.33          | 0.609      |
|               | 2   | 4.97 | 93.97        | 47.32           | 15.14           | 9.90  | 2.72          | 1.20          | 0.467      |
|               | 7   | 4.74 | 94.27        | 49.27           | 15.53           | 9.64  | 2.50          | 0.98          | 0.365      |
|               | 12  | 4.31 | 93.92        | 49.85           | 15.71           | 9.51  | 2.18          | 0.78          | 0.324      |
| SC            | 0   | 5.33 | 94.36        | 47.49           | 15.51           | 9.996 | 2.91          | 1.08          | 0.450      |
|               | 2   | 5.50 | 94.59        | 45.99           | 15.72           | 9.98  | 2.34          | 0.81          | 0.323      |
|               | 7   | 5.40 | 93.76        | 48.81           | 16.19           | 9.74  | 2.80          | 1.11          | 0.396      |
|               | 12  | 4.46 | 94.26        | 49.16           | 16.11           | 9.54  | 2.46          | 0.80          | 0.354      |
| LA + SC       | 0   | 6.04 | 94.40        | 46.14           | 14.05           | 10.66 | 3.50          | 1.32          | 0.607      |
|               | 2   | 4.96 | 92.60        | 50.38           | 15.19           | 10.04 | 2.90          | 1.22          | 0.466      |
|               | 7   | 4.52 | 94.22        | 51.37           | 15.62           | 9.66  | 2.35          | 0.81          | 0.323      |
|               | 12  | 4.19 | 93.26        | 52.84           | 15.85           | 9.55  | 2.09          | 0.74          | 0.296      |

**Table 1. The chemical composition of sesame meal before and after fermentation.**

* LA: *Lactobacillus acidophilus*; SC: *Saccharomyces cerevisiae*. 

*a,b*Means with no common superscripts within the column of each classification are significantly (*p* < .05) different, and each value represents the mean of four replicates.
Table 2. Amino acid profile of Sesame meal before and after fermentation by combination of Lactobacillus acidophilus and Saccharomyces cerevisiae after 12 days.

| Amino acids | RSM | FSM | SEM | p Value |
|-------------|-----|-----|-----|---------|
| Methionine  | 1.03| 1.17| 0.056| .2470 |
| Cysteine    | 0.43b| 0.52a| 0.026| .483 |
| Lysine      | 0.85b| 1.11a| 0.064| .0097 |
| Threonine   | 1.34| 1.40| 0.023| .2729 |
| Isoleucine  | 1.88| 1.96| 0.038| .2476 |
| Histidine   | 1.04| 1.06| 0.016| .4396 |
| Valine      | 1.99b| 2.21a| 0.061| .0394 |
| Leucine     | 2.99b| 3.89a| 0.205| .0006 |
| Arginine    | 4.77b| 5.19a| 0.103| .0078 |
| Phenylalanine| 1.86| 1.94a| 0.020| .0210 |
| Tyrosine    | 1.47| 1.84a| 0.085| .0018 |
| Tryptophan  | 0.76| 0.78| 0.024| .7687 |
| Alanine     | 1.99b| 2.24a| 0.063| .189 |
| Aspartate   | 3.55b| 4.21a| 0.148| .0001 |
| Glutamate   | 8.54b| 10.37a| 0.451| .0116 |
| Glycine     | 2.06| 2.51a| 0.117| .0260 |
| Proline     | 1.37| 1.57a| 0.049| .0179 |
| Serine      | 1.76| 1.77| 0.056| .8530 |
| Total       | 39.73b| 45.82a| 1.366| .0001 |

Means with no common superscripts within the column of each classification are significantly (p < .05) different, and each value represents the mean of four replicates.

RSM: raw sesame meal; FSM: fermented sesame meal.

**Fermentation process**

LA (PTCC: 1643) was prepared from Persian Type Culture Collection of Iranian Research Organisation for Science and Technology (IROST), and SC (Saf-levure, Lesaffre Group, France) were purchased from local Market. In Fermentation process a completely randomised design with 3 x 4 factorial arrangement was used for microorganism comparison (LA, SC and SC + LA) and fermentation time courses (0, 2, 7 and 12 days). The FSM was prepared according to the method used by Hassaan et al. (2015). Firstly, the raw sesame meal (RSM) was provided by the local company (Arsham Company, Tehran, Iran) and grounded to pass through a 0.5-mm sieve, and stored at 4°C until laboratory analysis. Experimental diets and

**Bird and treatments**

Farm experimental procedures were conducted in accordance with animal ethics committee guidelines of Guilan university. 420-day-old male broilers (Ross 308) were supplied from the commercial hatchery, individually weighed and allocated to a completely randomised design experiment with 7 treatments and 5 replicates (12 birds per pen). Diets were offered ad libitum and water was freely available. The experimental treatments were designed as follows: basal diet based on soybean meal (SBM), SBM substituted with 15% and 25% raw sesame meal (RSM) with and without phytase enzyme [5000 FTU phyzyme XP/g (PHX)]; RSM15, RSM25, RSM15 + PHX, and RSM25 + PHX, and SBM substituted with 15 and 25% FSM; FSM15 and FSM25. The chemical composition of the starter (1–10 days), grower (11–24 days) and finisher (25–42 days) diets are shown in Table 3. All diets were fed in mash form and the experiment endured for 42 days.

**Performance parameters**

Body weights of the broiler chicken and their feed intakes (FI) were recorded weekly. Mortality was recorded daily, and body weight gain (BWG), FI, and feed conversion ratio (FCR) for d 21 were calculated. Any died bird was weighed and FCR were calculated by dividing total feed intake by weight gain of live plus died birds.

**Nutrient digestibility and digestive enzyme activities**

On day 42, the birds were fasted for 24 h and then allowed to consume the experimental diets for 3 days before the collection of faecal samples, 10 g celite as a source of acid insoluble ash (AIA) was added per kg of diet and content of the AIA in diet and faeces were measured according to the De Coca-Sinova et al. (2011). Then, faecal samples were pooled, lyophilised, ground to pass through a 0.5-mm sieve, and stored at 4°C until laboratory analysis. Experimental diets and
faecal samples were analysed as described by AOAC (2005); briefly dry matter content using oven-drying (Method no. 930.15), and phosphorus concentration using spectrophotometr (Method no. 965.17). All values are expressed on a dry matter basis. The following equation was used to calculate the digestibility coefficient:

\[
\text{Digestibility coefficient} = \frac{(\text{Nutrient/AIA)}_{\text{diet}} - (\text{Nutrient/AIA)}_{\text{faecal}}}{(\text{Nutrient/AIA)}_{\text{diet}}}
\]

Digestive enzyme activities were determined using two randomly selected chicks per pen; euthanized by cervical dislocation, and then the mixed small intestinal (duodenum, jejunum, and ileum) contents were sampled. The intestinal digesta samples were diluted, homogenised, and centrifuged using the protocol described by Jin et al. (2000). Afterwards, the supernatants were divided into small portions and stored at −80°C till enzyme assays. Amylase activity was measured using soluble starch as a substrate, as described by Bernfeld (1955). Briefly, the mixture of supernatant and substrate was incubated at 40°C for 1 h, and the reaction was stopped by adding 1 mL dinitrosalicylic acid solution, followed by heating in boiling water for 5 min and cooling to room temperature. Maltose was determined by staining and colour intensity was measured by staining and colour intensity was measured at 410 nm by spectrophotometer. The proteolytic activity was determined by staining and colour intensity was measured at 540 nm. One unit of α-amylase activity was defined as the amount of enzyme that produced one milligram of maltose per minute at 40°C. Protease activity was analysed using the method reported by Lynn and Clevette-Radford (1984). A 0.2% solution of azocasein (substrate) in 1 mL of 200 mM Tris Hydrochloride buffer, pH 7, was digested by enzyme at 37°C for 2 h. The reaction was stopped with trichloroacetic acid (1 mL), clarified by centrifugation, and supernatant liquid absorbance was measured at 410 nm by spectrophotometer. The proteolytic activity unit was defined as milligrams of azocasein degraded for two hours incubation at 38°C per mg of total intestinal digesta protein. 

Carcass characteristics, digestive tracts development and lymphoid organs

On day 42, two birds per pen with body weights closest to the mean weight of the same pen were

Table 3. Composition and calculated analysis of the different experimental diets.

| Ingredients, % | SBM | RSM15 | FSM15 | RSM25 | FSM25 | SBM | RSM15 | FSM15 | RSM25 | FSM25 | SBM | RSM15 | FSM15 | RSM25 | FSM25 |
|----------------|-----|-------|-------|-------|-------|-----|-------|-------|-------|-------|-----|-------|-------|-------|-------|
| Corn           | 54.58| 54.23 | 52.12 | 54.13 | 52.73 | 57.52| 60.45 | 52.04 | 60.36 | 58.21 | 62.17| 64.87 | 65.39 | 67.25 | 65.95 |
| Soybean meal (CP: 44%) | 39.31| 22.76 | 11.52 | 19.58 | 7.54  | 35.89| 19.70 | 7.76  | 16.53 | 3.20  | 30.95| 14.74 | 4.03  | 11.83 | 0.00  |
| Sesame meal (fermentation) | 0.30 | 0.30  | 0.30  | 0.30  | 0.30  | 0.30 | 0.30  | 0.30  | 0.30  | 0.30  | 0.30 | 0.30  | 0.30  | 0.30  | 0.30  |
| Sesame meal    | 0.17 | 1.64  | 1.53  | 1.19  | 0.86  | 1.55 | 1.45  | 1.28  | 1.00  | 0.60  | 1.34 | 1.23  | 0.69  | 0.81  | 0.48  |
| Monocalcium phosphate | 0.00 | 0.00  | 0.00  | 0.00  | 0.00  | 0.00 | 0.00  | 0.00  | 0.00  | 0.00  | 0.00 | 0.00  | 0.00  | 0.00  | 0.00  |
| Potassium carbonate | 0.01 | 0.05  | 0.10  | 0.10  | 0.20  | 0.01 | 0.05  | 0.10  | 0.10  | 0.20  | 0.05 | 0.10  | 0.10  | 0.20  | 0.05  |
| Calcium carbonate | 1.00 | 0.47  | 0.06  | 0.78  | 0.02  | 1.10 | 1.23  | 0.69  | 1.23  | 0.47  | 1.10 | 1.23  | 0.69  | 1.23  | 0.47  |
| Sodium bicarbonate | 0.05 | 0.15  | 0.20  | 0.10  | 0.20  | 0.05 | 0.15  | 0.20  | 0.10  | 0.20  | 0.05 | 0.15  | 0.20  | 0.10  | 0.20  |
| Salt            | 0.36 | 0.29  | 0.32  | 0.32  | 0.14  | 0.30 | 0.29  | 0.32  | 0.14  | 0.30  | 0.30 | 0.29  | 0.32  | 0.14  | 0.30  |
| α-Methionine    | 0.31 | 0.31  | 0.31  | 0.31  | 0.31  | 0.31 | 0.31  | 0.31  | 0.31  | 0.31  | 0.31 | 0.31  | 0.31  | 0.31  | 0.31  |
| Lys-HCl         | 0.22 | 0.61  | 0.65  | 0.92  | 0.22  | 0.22 | 0.61  | 0.65  | 0.92  | 0.22  | 0.22 | 0.61  | 0.65  | 0.92  | 0.22  |
| L-Thr           | 0.19 | 0.17  | 0.23  | 0.21  | 0.28  | 0.19 | 0.17  | 0.23  | 0.21  | 0.28  | 0.19 | 0.17  | 0.23  | 0.21  | 0.28  |
| Filler          | 0.30 | 0.30  | 0.30  | 0.30  | 0.30  | 0.30 | 0.30  | 0.30  | 0.30  | 0.30  | 0.30 | 0.30  | 0.30  | 0.30  | 0.30  |
| PHX (FTU/Kg)1   | 0.20 | 500   | 500   | 0     | 0     | 0    | 500   | 500   | 0     | 0     | 500  | 500   | 0     | 500   | 0     |

1Supplied per kg diet: vitamin A, 11,000 IU; vitamin D3, 5000 IU; vitamin E, 36.75 IU; vitamin K3, 3.4 mg; vitamin B1, 1.98 mg; vitamin B2, 5.25 mg; pantothenic acid, 10.5 mg; niacin, 31.5 mg; vitamin B6, 2.87 mg; folic acid, 1.2 mg; vitamin B12, 0.024 mg; biotin, 0.105 mg; choline, 800 mg; manganese, 120 mg; zinc, 100 mg; copper, 20 mg; iron, 1 mg; selenium, 0.3 mg; antioxidant, 100 mg.

2PHX: 5000 FTU phyzyme XP/g, since the diets containing RSM have the same formulation as RSM diets, to fit the table on the page they are not shown.

SBM: Soybean meal; FSM4: 25% Fermented sesame meal; RSM15: 15% Raw sesame meal.
selected, weighed, and euthanized by cervical dislocation. After removal of feathers, feet, and head the carcase yield was determined. Cut-up parts such as thigh, breast, and abdominal fat were weighed. In addition, the empty weight of intestinal, gizzard, different parts of small intestine, and caeca were recorded. Then pancreas, thymus, liver, spleen, and bursa of Fabricius were removed and cleaned from adherent tissues. The weight of the different organs was expressed as relative weights (g/kg live body weight).

Statistical analysis

The data of first experiment were analysed by two-way ANOVA to determine the main effects (microorganism and time) and their interaction using general linear models procedure of the SAS (2009). The data of second experiment were analysed by one-way ANOVA using general linear model procedure of SAS (2009). Differences between means were evaluated using Tukey’s multiple comparison test. The p-values of ≤.05 were considered statistically significant, whereas values of .05 < p < .10 were declared a near-significant trend.

Results

Chemical composition and amino acid profile

The influence of microorganism strain and fermentation period on the chemical composition of RSM is presented in Table 1. The main effects of microorganism and fermentation times were significant factors affecting pH and crude protein of FSM (p < .05), However, no significant microorganism × day interaction was found for pH and crude protein (p > .05). LA + SC had a higher percentage of crude protein and LA showed a lower pH compared to other treatments (p < .05). In addition, the dry matter of FSM was not influenced by fermentation (p > .05). Considering the percentage of ether extract and ash in fermented treatments, there were no significant differences between treatments (p > .05), though, fermentation time had a significant effect on the two indices mentioned (p < .05). However, no interaction between microorganism strain and period of fermentation was observed for ether extract and ash (p > .05). As the fermentation time increased, the percentage of crude fibre, phytic acid and oxalate of FSM significantly decreased (p < .05). While for microorganism × day interaction, no significant differences were found for the percentage crude fibre, phytic acid and oxalate. In addition, the lowest percentage of phytic acid and oxalate in the FSM was observed after 12 days.

Amino acid profile of RSM before and after 12 days fermentation by combination of LA and SC (LA + SC) is presented in Table 2. The mean values of methionine, threonine, isoleucine, histidine, tryptophan and serine of FSM were recorded as 1.17, 1.40, 1.96, 1.06, 0.78, and 1.77%, respectively, which indicate a marginal increase over the RSM; whereas, significant increase was found for cysteine, lysine, valine, leucine, arginine, phenylalanine, tyrosine, alanine, aspartate, glutamate, glycine, and proline in FSM compared to RSM (p < .05). In addition, aspartic acid (3.55 and 4.21%), glutamate (8.54 and 10.37%), and arginine (4.77 and 5.19%) were found to be the most abundant
Table 5. Effect of dietary treatments on faecal dry matter, crude protein and Phosphorus digestibility and digestive enzyme activity of intestinal digesta of broilers at 42 days of age.

| Treatments | Dry matter | Protein | Phosphorus | Amylase, U/g | Protease, U/mg |
|------------|------------|---------|------------|--------------|----------------|
| SBM        | 0.89       | 0.74    | 0.60       | 15.90        | 66.62          |
| RSM15      | 0.89       | 0.72    | 0.44       | 15.30        | 64.25          |
| RSM25      | 0.87       | 0.73    | 0.36       | 15.58        | 63.51          |
| RSM25+PHX  | 0.87       | 0.73    | 0.55       | 15.47        | 64.81          |
| FSM25      | 0.89       | 0.74    | 0.58       | 15.39        | 65.00          |
| FSM15      | 0.89       | 0.72    | 0.58       | 16.08        | 64.48          |
| FSM25      | 0.89       | 0.74    | 0.56       | 16.41        | 65.41          |
| SEM        | 0.004      | 0.006   | 0.019      | 0.365        | 0.522          |
| p Value    | .637       | .882    | .002       | .967         | .835           |
| SEM p      | .004       | .006    | .019       | .036         | .522           |

| p Value    | .637       | .882    | .002       | .967         | .835           |

a,bMeans with no common superscripts within the column of each classification are significantly (p < .05) different, and each value represents the mean of five replicates.

SBM: Soybean meal; FSM15: 15% Fermented sesame meal; RSM15: 15% Raw sesame meal; PHX: Phyzyme XP (5000 FTU/g).

amino acids in the raw and fermented seed flours, respectively. These findings indicate that, total amino acids content of FSM significantly increased compared to RSM (p < .05).

Performance

Results from feeding trial, showed that BWG and FCR of birds were significantly affected by dietary treatments (Table 4), however, no significant differences were observed in FI between treatments for the entire experimental period. From 1 to 10 days of age, inclusion of RSM25 to the diet decreased BWG and increased FCR compared to other diets except for RSM15 containing diet (p < .05).

From 11 to 24 days of age, BWG was reduced (p < .05) as a result of inclusion of RSM25, but other treatments showed no significant differences with diet containing SBM. In addition, FCR of broilers fed RSM25 was higher (p < .05) than other treatments except RSM15 and FSM15.

From 25 to 42 days of age, the chicks fed RSM25 had lower BWG and higher FCR (p < .05) than those fed SBM, while, other treatments had no significant difference in BWG and FCR with SBM treatment. Overall, the inclusion of RSM25 in the diet decreased BWG and subsequently increased FCR in the whole period of the experiment (1–42 days).

Nutrient digestibility and digestive enzyme activities

The effects of dietary treatments on the nutrient digestibility and intestinal digestive enzyme activities in broilers on day 42 are shown in Table 5. There was no significant difference in the digestibility of dry matter and protein between SBM diet and the other diets (p > .05), however, phosphorus digestibility was decreased (p < .05) by RSM25 compared to other treatments, except for RSM15. Interestingly, there is no significant difference between FSM and SMB containing diets. As shown in Table 4, compared to SBM diet, all treatments did not have a significant effect on amylase and protease activities on day 42 (p > .05).

Carcass characteristics, digestive tracts development and lymphoid organs

The effects of the experimental diets on carcass characteristics, digestive tracts development, and lymphoid organs are shown in Table 6. Compared to FSM and RSM+PHX diets, only broilers fed diet containing RSM25 showed a significant decrease in carcass yield and breast weight on day 42 (p < .05), though, no differences were recorded between other diets (p > .05). Birds that were received diet containing FSM25 showed a tendency towards a decrease in abdominal fat (p = .07). The relative weights of digestive organs (gizzard, liver, pancreas, intestinal, duodenum, jejunum, ileum and cecum) and lymphoid organs (thymus, bursa and spleen) were not affected by dietary treatments (p > .05).

Discussion

Chemical composition and amino acid profile

The present investigation was intended to assess the effectiveness of LA and SC to improve the nutritive value of RSM under different fermentation periods. During the fermentation, the chemical composition of RSM as a result of metabolic activity of LA and SC was changed. As shown in Table 1, obviously, increasing the day of fermentation process resulted in a decrease in pH, phytic acid, oxalate and crude fibre, while the crude protein increased in FSM and the best results obtained after 12 days of fermentation. Another point to note is that, despite numerically reduction in pH, phytic acid, and oxalate content, it was not possible to estimate that the LA + SC treatment was more effective or that each of them alone had an effect to reduce, pH, phytic acid, oxalate and crude fibre. However, the main effects of LA + SC showed a significant increase in crude protein compared to other treatment. In the previous studies, a remarkable variation was reported in chemical compositions of FSM, partly due to the differences in the processing methods (mechanical press or solvent extraction) during the oil extraction step from the sesame seeds and duration of fermentation process. However, a similar trend
Table 6. Effect of experimental treatment on the relative weight of digestive and Lymphoid organs and Carcase [(g/kg body weight) × 100] of broilers.

| Treatment | Carcase, % | Digestive organs, % | Lymphoid organs, % |
|-----------|-----------|---------------------|---------------------|
|           |          | Gizzard | Liver | Pancreas | Intestinal | Duodenum | Jejunum | Ileum | Caeca | Thymus | Bursa | Spleen |
| SBM       | 65.66ab  | 22.60   | 26.33 ab | 2.75 | 0.65 | 1.09 | 1.02 | 0.20 | 0.19 | 0.18 | 0.15 |
| RSM15     | 64.26ab  | 21.03   | 26.36ab  | 2.89 | 0.68 | 1.19 | 1.09 | 0.22 | 0.18 | 0.18 | 0.16 |
| RSM25     | 60.65ab  | 22.71   | 23.38ab  | 2.90 | 0.65 | 1.16 | 1.13 | 0.24 | 0.17 | 0.18 | 0.15 |
| RSM15+PHX | 67.98ab  | 23.71   | 28.51ab  | 2.87 | 0.65 | 1.17 | 1.05 | 0.22 | 0.19 | 0.19 | 0.16 |
| RSM25+PHX | 67.71ab  | 23.06   | 27.80ab  | 2.82 | 0.64 | 1.17 | 1.01 | 0.20 | 0.18 | 0.18 | 0.16 |
| FSM15     | 67.71ab  | 22.86   | 27.38ab  | 2.76 | 0.64 | 1.14 | 0.99 | 0.21 | 0.17 | 0.18 | 0.16 |
| FSM25     | 68.05ab  | 22.87   | 27.08ab  | 2.79 | 0.60 | 1.17 | 1.02 | 0.21 | 0.17 | 0.17 | 0.15 |
| RSM15     | 66.06ab  | 0.291   | 0.377    | 0.025 | 0.045 | 0.031 | 0.013 | 0.015 | 0.01 | 0.004 | 0.003 | 0.002 |
| RSM25     | 64.44ab  | 0.291   | 0.377    | 0.025 | 0.045 | 0.031 | 0.013 | 0.015 | 0.01 | 0.004 | 0.003 | 0.002 |

*Means with no common superscripts within the column of each classification are significantly (p < 0.05) different, and each value represents the mean of five replicates.

has been reported in terms of RSM compounds in the fermentation process. The results of some studies indicate that combination of LA and fungi significantly decreased the pH of the fermented rapeseed meal (Chiang et al. 2009; Ashayerizadeh et al. 2017). In another study, where wheat flour and whole barley were incorporated into the fermentation process, a similar decrease in pH was observed, while lactic acid bacteria population increased (Skrede et al. 2003). The use of the mixture of LA and SC in the present study seems to create an environment where the SC consumes the oxygen present in the bag (Chiang et al. 2009) and subsequently provides a better environment for LA to produce organic acids resulting in a decrease in the pH of FSM (Van Winsen et al. 2001; Canibe et al. 2008; Chamberlain et al. 2019).

Furthermore, it has been shown that the fermentation of RSM for 15 days using Bacillus licheniformis decreased the crude fibre and phytic acid, while it increased the crude protein content (Roy et al. 2014). Olude et al. (2016) noted that fermentation of RSM by Lactobacillus plantarium for 48 h, increased the crude protein from 18.02% to 24.43%. They also reported that the fermentation process decreased the crude fibre and phytic acid contents of FSM from 37.2 to 22 g/kg and 7.2 to 6 g/kg, respectively. The fermentation of RSM by Lactobacillus acidophilus slightly increased the protein and lipid contents and decreased the phytic acid (Mukhopadhyay and Ray 1999a). Results from another study showed that the crude fibre, phytic acid and oxalate contents of fermented sesame seed decreased by 46, 50 and 69% respectively, while the crude protein and fat content increased marginally at the end of 96 h of fermentation (Olagunju and Ifesan 2013). Results obtained in this study showed that fermentation of RSM by LA + SC for 12 days leads to an increase in amino acid content. It has been demonstrated that amino acid contents increase by increasing the crude protein in the fermented soybean meal (Chen et al. 2010). Olagunju and Ifesan (2013) reported that aspartic acid, glutamic acid, and arginine were the most abundant amino acids in unfermented and fermented sesame flour, which agrees with the results of this study. The increase in protein content can be attributed to microbial synthesis of proteins, secretion of enzymes, and other biological products during fermentation by microorganisms (Elyas et al. 2002; Zhang et al. 2007). On the other hand, observed decrease in fibre content during fermentation by LA + SC treatment could be attributed to the partial solubilisation of cellulose and hemicellulosic type of material by microbial enzymes (Olagunju and Ifesan 2013).

The reasons for the low level of phytic acid content of FSM could be related to the enzymatic degradation of phytic acid by Lactobacillus spp. and S. cerevisiae that have been previously reported (Olukomaiya et al. 2020; Onipede et al. 2020). Furthermore, the enzymatic degradation of phytic acid by phytase enzyme requires an optimum pH which can be provided by natural fermentation (Gupta et al. 2015). The findings of the present study indicate the influence of fermentation on oxalate degradation, which is consistent with the results of the previous studies using L. acidophilus (Hatch 2017; Chamberlain et al. 2019). There is also evidence about an enzyme, known as S. cerevisiae acyl-activating enzyme 3 (ScAAE3), that catalyses the conversion of oxalate to oxalyl-CoA and reducing the inhibitory effects of oxalate on growth (Foster and Nakata 2014).

**Performance**

Beneficial effects of the phytase supplementation on the performance of broilers are well known and proven that phytase can increase the availability of several nutrients in diets (Simons et al. 1990; Gordon and Roland 1998; Kaneko et al. 2002; Shirley and Edwards 2003).
et al. (2006) reported that in corn-soybean meal-broiler diets containing 4.5 g/kg non-phytate phosphorus, increased ileal phosphorus digestibility by 14.7% in broilers. It has been reported that phytase phosphorus digestibility in broilers has been frequently demonstrated. The capacity of phytase to increase the total phosphorus digestibility in broilers has been frequently demonstrated. It has been reported that phytase increased ileal phosphorus digestibility by 14.7% in broiler diets containing 4.5 g/kg non-phytate phosphorus (Ravindran et al. 2000). In addition, Ravindran et al. (2006) reported that in a corn-soybean meal-based diet the ileal digestibility of phosphorus was decreased by increasing the phytate concentration and increased with increasing phytase content. The results of another study showed that adding phytase to the piglet diet containing RSM, did not affect apparent ileal digestibility of dry matter, crude protein, and energy digestibility but significantly improved the apparent ileal digestibility of calcium and phosphorus (de Souza et al. 2017). The phytase enzymes function to hydrolyse phytate and consequently release of phosphorus and other nutrients are well proved in many studies (Jendza et al. 2006; Selle and Ravindran 2007; Adeola 2010; Kong and Adeola 2011). To the best of our knowledge, no studies have reported concerning the effects of FSM on the digestibility of broiler chickens. However, available literatures have provided some reports concerning the effect of Lactobacillus spp. and Saccharomyces spp. to reduce phytate content of oilseeds and their meal during the fermentation process. For example, during starter phase, fermented rapeseed meal by a mixed liquid culture containing Lactobacillus fermentum, Enterococcus faecium, Saccharomyces cerevisiae, and Bacillus subtilis increased the apparent digestibility of dry matter and phosphorus compared to unfermented rapeseed meal in broiler chickens (Chiang et al. 2009). A possible explanation for the significant increase in phosphorus digestibility observed in FSM-fed broilers in the present study could be related to phytase activities of Lactobacillus spp. (Taheri et al. 2009; Garcia-Mantrana et al. 2016; Priyodip et al. 2017) and SC (Kłosowski et al. 2018) which have been previously reported. It is widely acknowledged that decomposition of phytic acid, increases the availability of many cations and followed by nutritional value (West 2014; Gupta et al. 2015).

Carcase characteristics, digestive tracts development and lymphoid organs

Results recorded for live weight and carcase weight indicated that the addition of RSM leads to decrease of these indices which is in agreement with findings of Rahimian et al. (2013) and David and John (2015). Another report showed that dressing percentage and slaughter weight were not affected by 9 and 18% RSM, while inclusion of 36% RSM resulted in a decrease (Shanti et al. 2012). They also showed that RSM and phytase interaction resulted in heavier giblets in birds fed with 36% RSM supplemented with 600 FTU/kg phytase. In addition, it has been showed that as the dietary RSM level increased, the dressing

Nutrient digestibility and digestive enzyme activities

Phosphorus digestibility in chicks receiving different levels of RSM + PHX and FSM significantly improved, and it was similar with those fed SBM diet (Table 4). The capacity of phytase to increase the total phosphorus digestibility in broilers has been frequently demonstrated. It has been reported that phytase increased ileal phosphorus digestibility by 14.7% in broiler diets containing 4.5 g/kg non-phytate phosphorus (Ravindran et al. 2000). In addition, Ravindran et al. (2006) reported that in a corn-soybean meal-based diet the ileal digestibility of phosphorus was decreased by increasing the phytate concentration and increased with increasing phytase content. The results of another study showed that adding phytase to the piglet diet containing RSM, did not affect apparent ileal digestibility of dry matter, crude protein, and energy digestibility but significantly improved the apparent ileal digestibility of calcium and phosphorus (de Souza et al. 2017). The phytase enzymes function to hydrolyse phytate and consequently release of phosphorus and other nutrients are well proved in many studies (Jendza et al. 2006; Selle and Ravindran 2007; Adeola 2010; Kong and Adeola 2011). To the best of our knowledge, no studies have reported concerning the effects of FSM on the digestibility of broiler chickens. However, available literatures have provided some reports concerning the effect of Lactobacillus spp. and Saccharomyces spp. to reduce phytate content of oilseeds and their meal during the fermentation process. For example, during starter phase, fermented rapeseed meal by a mixed liquid culture containing Lactobacillus fermentum, Enterococcus faecium, Saccharomyces cerevisiae, and Bacillus subtilis increased the apparent digestibility of dry matter and phosphorus compared to unfermented rapeseed meal in broiler chickens (Chiang et al. 2009). A possible explanation for the significant increase in phosphorus digestibility observed in FSM-fed broilers in the present study could be related to phytase activities of Lactobacillus spp. (Taheri et al. 2009; Garcia-Mantrana et al. 2016; Priyodip et al. 2017) and SC (Kłosowski et al. 2018) which have been previously reported. It is widely acknowledged that decomposition of phytic acid, increases the availability of many cations and followed by nutritional value (West 2014; Gupta et al. 2015).

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proportion was decreased (Kaneko et al. 2002). Al Harthi and El Deek (2009) demonstrated that lower dosage inclusion of RSM (5, 10 and 15%) with and without phytase in diet of broilers did not have significant effects on the carcase, breast, liver, heart, thigh, and gizzard. Similarly, it has been reported that there are no significant differences on carcase dressing percentage and meat in broilers fed with 5 and 10% RSM compared with control diet (Nikolakakis et al. 2014). Regarding the abdominal fat, it has been shown that the abdominal fat percentage was decreased by feeding RSM and phytase enzyme (Rahimian et al. 2013). Also, relative weights of abdominal fat were not affected by inclusion of RSM up to 0.67 of SBM, but it decreased significantly with further increase in RSM up to 1.00 of SBM (Rama Rao et al. 2008). However, in this research, there was a tendency towards decreasing abdominal fat by FSM25. Also, the percentage of the abdominal fat was numerically decreased by increasing the level of RSM. Reduced feed efficiency and poor availability of protein and phosphorus (Tables 2 and 4) in broilers receiving RSM as a substitute for SBM might be responsible for low carcase yield. Based on findings in this study, adverse effects of RSM could be eliminated by fermentation process. As shown in Table 4, not only the carcase yield of the birds fed with two levels of FSM did not significant differences with control and phytase containing diets but also there was a tendency to decrease the abdominal fat.

Conclusions

The in vitro results indicated that, increasing the time course of fermentation, the availability of nutrients in FSM increased, and the best results obtained after 12 days. However, in concern to availability of nutrients in FSM among treatments, clear differences have not obtained between the individual and combinatorial effects of the LA and SC. Yet, the main effects of the microorganism to increase crude protein was significant for LA + SC treatment. The in vivo results showed that the FSM and RSM + PHX diets improved phosphorus digestibility and subsequently performance of broilers as same as SBM diets. Based on the findings of this study, diets containing different levels of RSM25 reduced BWG and impaired the growth compared to other diets. All in all, more detailed studies are needed to completely remove of oxalate and phytic acid in RSM.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are available from the corresponding author, Majid Mottaghitalab, upon reasonable request.

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