



The Effect of Fermented Sesame Meal or its Combination with Probiotics on the Performance, Carcass Traits, Blood Parameters, and Humoral Immunity in Growing Japanese Quails

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Abstract

In this experiment, the effect of fermented sesame meal or its combination with probiotics was evaluated on performance, carcass traits, blood parameters, and humoral immunity of Japanese quails. In a completely randomized design, 480 seven-day-old Japanese quails were assigned to eight treatments, four replicates, and fifteen chicks per replicate. Treatments were corn-soybean meal diet as a basal or control group (1), and basal diet containing 35% of sesame meal (2), 35% of fermented sesame meal with *Saccharomyces cerevisiae* (3), 35% of sesame meal + *Saccharomyces cerevisiae* probiotic (4), 35% of fermented sesame meal with *Bacillus subtilis* (5), 35% of sesame meal + *Bacillus subtilis* probiotic (6) 35% of sesame fermented meal with *Lactobacillus sakei* (7), and 35% of sesame meal + *Lactobacillus sakei* probiotic (8). The study results indicated that treatments with sesame meal, compared to control treatment, had no significant effects on the performance and blood parameters of Japanese quails. Birds fed with a diet containing 35% of fermented sesame meal with *Saccharomyces cerevisiae* had a lower relative liver weight than the control treatment, whereas birds fed with a diet containing 35% sesame meal plus *Lactobacillus sakei* probiotic had a greater relative liver weight ($P < 0.05$). Treatment with fermented sesame meal with *Saccharomyces cerevisiae* had a higher level of antibody produced against sheep red blood cells and Newcastle virus than those of the control group ($P < 0.05$). Findings revealed that up to 35% of sesame meal not only does not impair the performance of Japanese quails, and fermenting of this protein supply but can also improves the birds' health.

Introduction

Today, the greatest challenge faced by the poultry industry in Iran is the shortage of feed items needed to formulate the diet. Therefore, serious efforts should be made to use domestic feed sources which possess a good nutritional value. According to the Food and Agriculture Organization (FAO), about 10.6 million tons of sesame is produced annually in the world, of which Iran's share of this production is approximately 31,000 tons (FAO, 2019). Sesame (*Sesamum indicum* L.) belongs to the Pedaliaceae family, is unique to tropical and subtropical regions (Kindeya *et al.*, 2019). Sesame cultivation due to the

drought tolerance, farmers' familiarity with the cultivation of this plant, the possibility of cultivating it after harvesting wheat and barley, as well as the high quality of its oil have long been common in Iran (Ahmadi *et al.*, 2000).

Sesame seeds contain 45-50% oil, 15-24% protein, 10-15% carbohydrates, and about 1.5% lignans (sisamulin and sisamine) (Borchani *et al.*, 2010). The potent antioxidant properties of sesame seeds are attributed to lignans, a type of phytoestrogen (Park *et al.*, 2010). The sesame meal resulting from the seeds after oil extraction is an inexpensive source of protein that contains fibers and

chemical compounds such as phenolic antioxidants and approximately 44% of crude protein (Vioque *et al.*, 2000). Since sesame meal is rich in methionine, its concomitant use with soybean meal, which is rich in lysine, can create a good amino acid balance in the diet (Farran *et al.*, 2000).

Previous studies evaluated the possibility of dietary supplementation of sesame meal. For instance, up to 20% of sesame meal in Japanese quail diets had no adverse effect on daily weight gain, feed conversion ratio, and feed intake (Ghazvinian *et al.*, 2016). In the other study, using 18% of sesame meal did not show any negative effect on the blood parameters of broilers. However, some researchers indicated that the inclusion of sesame meal in the starter diet of broilers was limited due to the high amount of fiber and probable digestive difficulties with oxalate and phytate (Yakubu and Alfred, 2014).

In recent years, functional feeds produced by the fermentation process have been employed to improve the growth performance, immune system, and gastrointestinal health of poultry (Alshelmani *et al.*, 2016; Sugiharto and Ranjitkar, 2019). Fermented feeds contain high levels of lactic acid bacteria, low pH, and high concentrations of organic acids, which not only improve the performance and health of poultry, but also reduce feed contamination to pathogens before consumption (Niba *et al.*, 2009; Xie *et al.*, 2016). Khempaka *et al.* (2013) reported that the fermentation process with the help of different bacterial and fungal species improved the quality of protein products, reduced the extent of antinutritional compounds, and enhanced the digestive system health. Meal fermentation has been reported to have many advantages, like allergen alteration during fermentation by proteolytic enzymes. An effective fermentation process removes antinutritional compounds and also enhances the nutritional value of feed (Khempaka *et al.*, 2013).

Probiotics ameliorate growth performance, enhance nutrient digestibility (Cheema *et al.*, 2003), and help the consumption of non-digestible carbohydrates (Khempaka *et al.*, 2013). *Saccharomyces cerevisiae*, as a probiotic in the feed, could improve weight gain, feed efficiency (Kapila & Sinha, 2006), and immune responses of broilers (Song *et al.*, 2008). Likewise, *Lactobacillus*-based probiotics enhance the growth performance through increasing nutrient absorption and microbial balance in the digestive system of broilers (Yusrizal & Chen, 2003). In addition, probiotics based on *Bacillus* have higher resistance against unfavorable peripheral circumstances such as temperature and acidity due to sporulation. In addition, *Bacillus*-based probiotics have higher compatibility with the interior of the digestive system than *Lactobacillus*-based probiotics (Cheema *et al.*, 2003). In recent years, the supply of soybean meal has been one of the main problems of

the poultry industry in Iran. The present research aimed to examine the effect of reducing soybean meal usage in the diet by using sesame meals. Additionally, the effects of this protein source on the performance features, Carcass properties, blood parameters, and humoral immunity of Japanese quail were further evaluated.

Materials and Methods

Birds, experimental design, and feed preparation

The Research Animal Ethics Committee of the University of Zabol approved the experimental procedures before the commencement of the trial. 480 seven-day-old Japanese quails (males and females) were assigned in a completely randomized design to eight treatments with four replicates, and fifteen chicks per replicate. Treatments were corn-soybean meal diet as a basal or control group (1), and basal diet containing 35% of sesame meal (2), 35% of fermented sesame meal with *Saccharomyces cerevisiae* 35% of sesame meal + *Saccharomyces cerevisiae* probiotic, 35% of fermented sesame meal with *Bacillus subtilis* (5), 35% of sesame meal + *Bacillus subtilis* probiotic (6) 35% of sesame fermented meal with *Lactobacillus sakei* (7), and 35% of sesame meal + *Lactobacillus sakei* probiotic (8).

In the present study, *Saccharomyces cerevisiae* yeast (PTCC 5269, lyophilized vials, obtained from the fungi and bacteria collection center of the Iranian Research Organization for Science and Technology), was and *Lactobacillus Sakei* bacteria (IBRC-M 10666, provided by the National Center for Genetic and Biological Resources of Iran) were activated by yeast extract peptone (YPD) broth and nutrient broth culture media at 37 °C, respectively. Primer culture media for yeasts and bacteria were provided through YPD agar and De Man, Rogosa, and Sharpe (MRS) broth culture media and incubated at 30 and 37° C, respectively. The fermentation process was conducted according to the methods developed by Feng *et al.* (2007) and Shi *et al.* (2017) with minor modifications. In brief, sterile water was added to the milled sesame meal (~1:3, v:v), to obtain 40% moisture content and autoclaved for 15 minutes at 121 °C. The autoclaved sesame meal was cooled at room temperature and inoculated and incubated under aerobic conditions at 37°C for 24 h with *Saccharomyces cerevisiae* and *Bacillus subtilis*, and under anaerobic conditions at 37°C for 48 hours with *Lactobacillus sakei* (at a density of 10⁸ CFU per gram of sesame meal). Finally, the fermented sesame meal was dried in the oven at 50°C for three days. *Saccharomyces cerevisiae* probiotic (Iran-Melas Company, Fariman, Iran) was used at a ratio of 2 g (2.5 × 10⁸ cfu/g of yeast) per kg of the diet, and *Bacillus subtilis* probiotic (Calsporin®) was used at a ratio of 50 mg (1.0 × 10¹⁰ cfu/g of *B. subtilis*) per kg

of feed. To add *Lactobacillus sakei* to the diet, the strain was cultured in sterilized MRS broth twice in a row. The produced biomass was separated from the culture medium by centrifuging at $11,200 \times g$ for 10 minutes at 4°C . The acquired pellet was washed again with sterile distilled water and separated from water at $11,200 \times g$ for 5 minutes at 4°C . Finally, after freeze-drying, it was added to the diet at a ratio of 50 mg/kg (1.0×10^{10} cfu/g of *L. sakei*). All three probiotics used in this study provided a concentration

of 5.0×10^8 CFU microorganisms per kg of feed. Soybean meal, sesame meal, and fermented sesame meal samples were analyzed for protein and amino acid content through near-infrared reflectance spectroscopy (NIRS) procedure in Degussa, Germany. The diets were managed for a period of 7 to 35 days according to the nutritional requirement recommended by the National Research Council (NRC, 1994; Table 1). During the rearing period, the chickens had free access to feed and water.

Table 1. Chemical composition and calculated analysis of the control and experimental diets

Item	Corn-soybean diet (control)	Sesame meal diet (SM)
Ingredient (%)		
Corn	52.51	40.32
Soybean meal (43.8%)	35.00	9.87
Sesame meal (39.41%)	0.00	35.00
Soybean oil	1.16	1.40
Corn gluten meal (62%)	8.00	8.00
Calcium carbonate	1.42	2.26
Sodium bicarbonate	0.25	1.75
L-lysine HCL	0.10	0.70
DL-Methionine	0.21	0.02
L-Threonine	0.00	0.10
Dicalcium phosphate	0.68	0.00
Vitamin Premix ¹	0.25	0.25
Mineral Premix ²	0.25	0.25
Sodium chloride	0.17	0.08
Chemical analysis ³ :		
ME (kcal/kg diet)	2950	2950
Crude protein (%)	25.87	25.87
Lysine (%)	1.30	1.30
Methionine (%)	0.63	0.63
Methionine+Cystine (%)	1.05	1.05
Threonine (%)	0.97	0.97
Valine (%)	1.21	1.15
(Na + K)-CL mEq/kg	250	250
Calcium (%)	1.59	1.59
Available Phosphorus (%)	0.56	0.56

¹Vitamin premix provided per kilogram of diet: vitamin A (vitamin A acetate), 11,500 IU; cholecalciferol, 2,100 IU; vitamin E (dl- α -tocopheryl acetate), 22 IU; vitamin B12, 0.60 mg; riboflavin, 4.4 mg; nicotinamide, 40 mg; calcium pantothenate, 35 mg; menadione (menadione dimethyl-pyrimidinol), 1.50 mg; folic acid, 0.80 mg; thiamine, 3 mg; pyridoxine, 10 mg; biotin, 1 mg; choline chloride, 560 mg; and ethoxyquin, 125 mg.

²Mineral premix provided per kilogram of diet: Mn ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$), 65 mg; Zn (ZnO), 55 mg; Fe ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), 50 mg; Cu ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), 8 mg; I [$\text{Ca}(\text{IO}_3)_2 \cdot \text{H}_2\text{O}$], 1.8 mg; Se, 0.30 mg; Co (Co_2O_3), 0.20 mg; and Mo, 0.16 mg.

³Calculated according to NRC (1994).

Performance parameters and carcass processing

Performance parameters such as body weight, weight gain, feed intake, and feed conversion ratio were measured at 35 d. At the end of the experimental period, two birds with a weight close to the average weight of the corresponding experimental unit were selected and euthanized by cervical dislocation for carcass processing. The relative weight of different sections was calculated as the percentage of body weight.

Blood biochemical parameters

At the end of the experimental period, four birds were selected from each cage, and blood samples were

taken from the wing vein. To separate the serum, the blood samples were retained at room temperature for two hours. The blood samples were centrifuged at $1,008 \times g$ for 15 minutes; the resulting serum samples were poured into micro tubes and preserved at -20°C until the biochemical analysis. Blood parameters including glucose, cholesterol, triglycerides, total protein, albumin, and aspartate aminotransferase (AST) of the serums were measured using commercial kits (Parsazmun, Tehran, Iran) according to their standard procedures and via an auto-analyzer machine (Alcyon 300, Abbot Park, IL., USA).

Humoral immunity

On 21 and 28 days of age, 0.2mL of sheep red blood cell (SRBC) antigen (5%) was injected into the breast muscle. Seven days after the injections, blood sampling was performed through the wing vein to assess the primary and secondary responses to SRBC. Vaccination against Newcastle disease virus (NDV) was performed on day 18 using the lyophilized vaccine (Live B₁ strain; Vetrina; Zagreb, Croatia) through eye drops. On day 35, blood sampling was performed from four birds of each replicate and the antibody produced against antigens was measured by a hemagglutination inhibition test in the serum

samples (Cheema *et al.*, 2003).

Statistical analysis

The obtained data were analyzed by a general linear model for a completely randomized experimental design using the SAS software (SAS, 2002), and the means were compared by Tukey's test ($P < 0.05$).

Results

Performance parameters

Productive performance of birds including body weight, weight gain, feed intake, and feed conversion ratio are shown in Table 2. None of the mentioned parameters were significantly different ($P < 0.05$).

Table 2. Effect of dietary treatments on body weight, body weight gain, feed intake, and feed conversion ratio of growing Japanese quails from 7 to 35 days of age

Treatments	BW ¹ (35 d)	BW gain (g)	Feed intake (g)	FCR ²
Control (corn-soybean meal diet)	154.20	129.70	447.60	3.68
Diet containing 35% sesame meal	150.80	126.20	475.00	3.76
Diet containing 35% fermented sesame meal with <i>Saccharomyces cerevisiae</i>	157.60	133.00	490.10	3.68
diet containing 35% sesame meal + <i>Saccharomyces cerevisiae</i> probiotic	161.70	137.20	485.80	3.54
diet containing 35% fermented sesame meal with <i>Bacillus subtilis</i>	153.20	128.70	474.90	3.69
diet containing 35% sesame meal + <i>Bacillus subtilis</i> probiotic	157.90	133.40	514.20	3.86
diet containing 35% fermented sesame meal with <i>Lactobacillus sakei</i>	152.00	127.50	488.10	3.83
diet containing 35% sesame meal + <i>Lactobacillus sakei</i> probiotic	158.90	134.40	505.90	3.77
SEM ³	2.790	2.790	11.180	0.09
P-value	0.121	0.119	0.164	0.378

¹ BW: body weight

² FCR: feed conversion ratio

³ SEM: Standard error of the means

Table 3. Effect of dietary treatments on the carcass and internal organs of growing Japanese quails (% of live body weight)

Treatments	Carcass yield	Liver	Heart	Gizzard	Spleen	Bursa of Fabricius
Control (corn-soybean meal diet)	62.80	3.23 ^a	0.71	2.08	0.08	0.13
Diet containing 35% sesame meal	62.30	3.27 ^{ab}	0.74	2.21	0.11	0.13
Diet containing 35% fermented sesame meal with <i>Saccharomyces cerevisiae</i>	63.50	2.28 ^b	0.72	1.97	0.08	0.1
diet containing 35% sesame meal + <i>Saccharomyces cerevisiae</i> probiotic	63.30	2.61 ^{ab}	0.85	1.94	0.09	0.11
diet containing 35% fermented sesame meal with <i>Bacillus subtilis</i>	63.30	2.51 ^{ab}	0.87	2.01	0.06	0.11
diet containing 35% sesame meal + <i>Bacillus subtilis</i> probiotic	62.60	2.46 ^{ab}	0.80	1.77	0.07	0.13
diet containing 35% fermented sesame meal with <i>Lactobacillus sakei</i>	62.40	2.64 ^{ab}	0.76	2.18	0.07	0.11
diet containing 35% sesame meal + <i>Lactobacillus sakei</i> probiotic	60.60	3.31 ^a	0.80	1.98	0.08	0.14
SEM ¹	1.32	0.20	0.04	0.14	0.01	0.01
P-value	0.845	0.008	0.160	0.510	0.500	0.730

^{a-b} Means not sharing a common superscript in a column are significantly different ($P < 0.05$).

¹ SEM: Standard error of the means

Carcass attributes

Table 3 presents the effects of different treatments on carcass yield and the relative weight of internal organs. Except for the liver weight, other parameters were not significantly affected. Birds fed diet containing 35% fermented sesame meal with *Saccharomyces cerevisiae* had a lower relative liver

weight than the control treatment.

Blood parameters

Table 4 presents the effects of experimental treatments on blood parameters. The sesame meal and the additives did not significantly affect the serum glucose, cholesterol, triglycerides, total protein, albumin, and AST levels.

Table 4. Effect of dietary treatments on some blood variables of growing Japanese quails

Treatments	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	AST ¹ (U/L)
Control (corn-soybean meal diet)	373.0	250.2	459.5	5.4	3.7	280.2
Diet containing 35% sesame meal	363.0	245.2	461.7	5.6	3.7	287.7
Diet containing 35% fermented sesame meal with <i>Saccharomyces cerevisiae</i>	257.0	230.0	434.7	6.2	3.8	280.5
diet containing 35% sesame meal + <i>Saccharomyces cerevisiae</i> probiotic	388.2	248.5	472.0	5.5	3.6	276.5
diet containing 35% fermented sesame meal with <i>Bacillus subtilis</i>	371.2	260.2	463.7	5.8	3.5	282.0
diet containing 35% sesame meal + <i>Bacillus subtilis</i> probiotic	370.0	249.5	487.2	5.5	3.3	286.0
diet containing 35% fermented sesame meal with <i>Lactobacillus sakei</i>	367.2	232.5	440.5	5.9	3.7	283.0
diet containing 35% sesame meal + <i>Lactobacillus sakei</i> probiotic	368.5	261.2	478.7	5.5	3.5	282.0
SEM ²	10.58	8.88	12.86	0.2	0.11	4.71
P-value	0.620	0.175	0.109	0.186	0.102	0.789

¹ AST: Aspartate Aminotransferase

² SEM: Standard error of the means

Humoral Immunity

The effect of the experimental treatments on humoral immunity revealed that sesame meal and additives had no significant effect on the antibody produced in the first challenge with sheep red blood cells (Table 5). However, the humoral immune response showed a trend ($P = 0.088$) in the first challenge with SRBC, and treatments containing *L. Sakei* probiotic and fermented sesame produced more antibodies. Sesame

meal fermentation with *Saccharomyces cerevisiae* resulted in a higher antibody titer in the second challenge with SRBC and the challenge with NDV compared to the control group ($P < 0.05$). Moreover, in the second challenge with SRBC, the treatment containing fermented sesame produced more antibodies than the treatment containing *B. subtilis* probiotic.

Table 5. Effect of dietary treatments on humoral immunity of growing Japanese quails

Treatments	ANDV ¹ (log ₂)	ASRBC1 ²	ASRBC2 ³
Control (corn-soybean meal diet)	4.00 ^b	3.50	5.25 ^c
Diet containing 35% sesame meal	4.50 ^{ab}	5.00	6.75 ^{abc}
Diet containing 35% fermented sesame meal with <i>Saccharomyces cerevisiae</i>	6.75 ^a	5.25	8.25 ^a
diet containing 35% sesame meal + <i>Saccharomyces cerevisiae</i> probiotic	5.00 ^{ab}	4.50	6.25 ^{abc}
diet containing 35% fermented sesame meal with <i>Bacillus subtilis</i>	5.50 ^{ab}	4.25	6.25 ^{abc}
diet containing 35% sesame meal + <i>Bacillus subtilis</i> probiotic	5.25 ^{ab}	4.50	5.75 ^{bc}
diet containing 35% fermented sesame meal with <i>Lactobacillus sakei</i>	6.25 ^{ab}	4.75	6.75 ^{abc}
diet containing 35% sesame meal + <i>Lactobacillus sakei</i> probiotic	5.50 ^{ab}	5.50	7.75 ^{ab}
SEM ⁴	0.51	0.44	0.43
P-value	0.021	0.088	0.001

^{a-b} Means not sharing a common superscript in a column are significantly different ($P < 0.05$).

¹Antibody titer against Newcastle disease virus; ²Antibody titer against SRBC1; ³Antibody titer against SRBC2; ⁴Standard error of the means

Discussion

In this study, the performance of Japanese quail was not affected by sesame meal and additives consumption. In contrast, sesame meal was

considered a suitable alternative to soybean meal in the previous studies (Bell et al., 1990; Ngele et al., 2011). Interestingly, sesame meal, in other studies, reduced the feed intake and increased the feed

conversion ratio (Mamputu and Buhr, 1995; Ghazvinian *et al.*, 2017). A higher feed conversion ratio of chickens fed with sesame meal may contribute to more fiber content or the cyanide, oxalate, and trypsin inhibitors existing in sesame meal (Ghazvinian *et al.*, 2017). These varying results might be due to the difference in the bird species.

Consumption and processing of sesame meal had no significant effect on the carcass attributes except for the liver weight. The relative liver weight was significantly lower in the treatment containing fermented sesame meal with *Saccharomyces cerevisiae* than in the control treatment. Owing to microbial metabolism during fermentation, the concentration of some amino acids increases in fermented sesame meals (Niba *et al.*, 2009). Probably, by increasing the concentration of amino acids in the diet, the required amino acids are better provided and lipoproteins are released more conveniently from the liver, resulting in less fat accumulation in the liver (Iwao *et al.*, 2020). Active microbes during fermentation decrease the activity of the Acetyl-CoA carboxylase enzyme (the rate-limiting enzyme for the synthesis of fatty acids), thereby controlling the amount of fat production in the body and the liver (Santoso *et al.*, 1995). In similar studies, carcass attributes, including carcass percent, breast, thigh, gizzard, small intestine, and relative weight of proventriculus in treatments with sesame meal supplemented with phytase enzyme or probiotic, were not affected (Al Harthi and El Deek, 2009).

Sesame meal had no significant effect on blood parameters. Similar to the findings of the present study, the use of sesame meal alone, along with phytase enzyme or probiotic supplement, had no significant effect on blood parameters such as cholesterol and triglyceride in broilers (Al Harthi and El Deek, 2009). Yalçın *et al.* (2013) reported that probiotics have no effects on blood triglyceride and cholesterol concentrations in broilers and quails. However, some studies reported a reduction in blood triglyceride and cholesterol levels as a result of probiotic consumption or fermentation (Parvez *et al.*, 2006; Ooi and Liong, 2010). These contradictory results could be due to the type of meal processing,

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the percentage of the extracted oil, and the extraction temperature.

In the second challenge with SRBC and the challenge with Newcastle virus, the fermentation with *Saccharomyces cerevisiae* increased the antibody titer compared to the control treatment. One of the characteristics of fermented feeds is an increase in the number of lactic acid-producing bacteria and consequently a high concentration of lactic acid in the feed. Therefore, consumption of fermented feed improves the defense barriers in the birds' digestive system against pathogens through pH reduction (Niba *et al.*, 2009). Furthermore, the fermentation increases feed's bioactive peptides and improves the animals' immune system (Feng *et al.*, 2007; Hou *et al.*, 2017). Accordingly, it was reported that probiotics in broiler and layer diets increase the antibody titers against SRBC antigen (Panda *et al.*, 2000). Although the exact mechanism by which probiotics improve the immune response is still unclear, they could stimulate various immune cells to produce cytokines involved in inducing and regulating immune responses (Christensen *et al.*, 2002).

Conclusion

The present study revealed that the use of 35% unprocessed sesame meal in quails' diet had no negative effect on their performance. In addition, fermentation of the meal with *Saccharomyces cerevisiae* enhances Japanese quail's immune response. Due to the unavailability and high cost of soybean meal, it is recommended that sesame meal be used in the Japanese quails' diet to overcome soybean meal shortage, and microbial processing of the meal with *Saccharomyces cerevisiae* be employed to improve the birds' immunity response against viral diseases.

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Conflict of interest statement

There are no known conflicts of interest.

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