Influence of spleen meal and hydrolyzed yeast on growth performance, blood cells, antibody titres and IL-2 gene expression in broiler chickens

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ABSTRACT
Two experiments were conducted to evaluate spleen meal (SPM) nutritional value and compare its effects and hydrolyzed yeast (HY) in broiler chickens. In experiment 1, nutrient composition and metabolizable energy content of SPM were determined using male broilers. In experiment 2, 600 broiler chicks were assigned to 5 groups. Dietary treatments were control group, HY1: hydrolyzed yeast (1 g/kg), HY2: hydrolyzed yeast (2 g/kg), SPM1: (30 g/kg diet) and SPM2: (60 g/kg diet). Interleukin-2 (IL-2) gene expression and antibody titre against Newcastle and infectious bursal diseases were evaluated on day 21. Apparent metabolizable energy (AME) and AMEn content of SPM were 3496 and 3296 kcal/kg, respectively. Supplementation with SPM improved (P < 0.001) body weight gain and feed conversion ratio compared to control and HY1 groups. Antibody titre against IBV was improved in SPM1, SPM2 and HY2 groups compared to HY1 and control groups. WBC was increased and heterophils was reduced in groups fed with SPM compared to control and HY groups. The highest gene expression for IL-2 was observed in broilers which received SPM1 and SPM2 compared to control and HY groups. It was concluded that dietary supplementation of HY and SPM has potential to improve broiler performance and immune response.

Introduction
Using alternative protein sources to replace soybean meal can be an attractive strategy to decrease cost of diets and reduce soybean level in poultry diets (Rochell et al. 2011). It may also lead to better amino acid balance and improve bird performance and health. During early growth periods, high protein diets are necessary and are often achieved by higher inclusions of soybean meal, which contains oligosaccharides and antigenic proteins that are not well digested and can contribute to enteric issues (i.e. inflammation). The benefits would be higher if the supplements are of high quality, easily digestible, and bioavailable. Processing of cattle for their meat leads to enormous amounts of byproducts (Khalaji et al. 2016). Spleen is an organ of the lymphatic system and acts as primary filter for the blood and keeps the body fluids in balance. Tuftsin is an immune modulating tetrapeptide found in spleen (Najjar and Nishio 1970). In an animal model, it was reported that Tuftsin could enhance immunologic effects of cells of monocytic origin, thereby shows antimicrobial and antitumor activities (Liu et al. 2012; Gao et al. 2015).

A worldwide attempt has been executed to remove antibiotic as growth promoter in rations, because antibiotics have residues in poultry products, resulted in resistant bacteria and normal microflora imbalance in gut (Schjørring and Krogfelt 2011; Yoon and Yoon 2018). There are many alternatives for antibiotics including probiotics, prebiotics, and phytobiotics, as well as organic acids, essential oils, and enzymes (Saleh et al. 2020). Yeast-based prebiotics are also one of the alternatives which counter the growth of pathogenic bacteria and could enhance the immune response. One of the components in yeast-based prebiotics is mannana-oligosaccharide which exist in the cell surface of yeast and could act as high-affinity ligands. Thus, this component could exhibit an extent competitive binding site for most bacteria (Abudabos and Yehia 2013). In the surface of gram negative bacteria like E. coli and Salmonella, there is mannose-specific fimbriae which could attach to and colonize in the intestinal wall. These bacteria could also attach to mannana-oligosaccharide instead of intestinal surfaces and then removed from intestinal cavity (Shashidhara and Devegowda 2003; Yang et al. 2008; Kim et al. 2011; Saeed et al. 2017).

In the yeast-based prebiotics, there is also a component named β-glucans. The immune systems of birds could recognize β-glucan as a foreign component and in a number of disease challenge works has been shown to be a protective agent and immune enhancer (Chae et al. 2006; Zhang et al. 2008; Cox et al. 2010; Moon et al. 2016). In the literature, there are several studies that have shown growth promotion of broiler chicks by feeding yeast-based probiotics; however, there is limited information concerning the effects of spleen meal (SPM) and hydrolyzed yeast (HY) product on the immune response and interleukin-2 (IL-2) gene expression in broiler chickens.

Therefore, the present work was conducted to evaluate SPM nutritional value and compare the effects of feeding SPM and
HY on performance, blood attributes, antibody titres against Newcastle, and infectious bursal disease viruses and IL-2 gene expression in broiler chickens.

Materials and methods

Preparation of HY and SPM samples

The HY sample was prepared from Behan Kimia Enzyme Company (Tehran, Iran) and included in the broiler rations at the amount of 1.0 and 2.0 g/kg (0.1% and 0.2% as fed). The HY is rich in the amounts of mannan-oligosaccharide (13%) and β-glucan (18%) and has been produced from Saccharomyces cerevisiae. SPM was prepared from a slaughterhouse producing SPM and powder. Bovine SPM as powder was used in this study.

SPM chemical and metabolizable energy evaluation

DM, CP, EE, Ca, P, Na, K, Fe, Cu, and Zn content of SPM were measured by standard methods (AOAC 1994). The SPM sample was sent to AMINOLab (Evonik, Germany) for amino acids analysis using HPLC. DM, CP, EE, Ca, P, Zn, Fe, and Cu analyses of SPM were done by standard procedures (AOAC 1994). Metabolizable energy of the SPM was determined using total excreta collection in male broilers. On day 33 of, male broilers of age 48 were placed in battery cages and divided into two groups with four replicate and six birds in each replicate. One group was fed a basal corn soybean meal diet (ME, 3100 kcal/kg; CP, 19.3%) and the basal diet was replaced by 50% SPM in another group. On day 38, experimental feed and total excreta were measured for 72 h. Trays lined under the cages to prevent the loss of excreta. Feed and excreta dry matter, gross energy, and nitrogen were determined. Metabolizable energy of the basal diet and test diets was calculated. The apparent metabolizable energy (AME) of SPM was then calculated using the difference method (Tancharoenrat et al. 2013).

Birds and experimental design

One-day-old Cobb 500 broiler chicks (n = 600) were purchased from a commercial hatchery. In a completely randomized design, the chicks were randomly assigned into five treatment groups and housed in pens (1.3 m × 1.2 m) with wood shavings. Each treatment group had 6 replicates and each replicate had 20 chicks. The house temperature in the first week was kept at 33°C and decreased to 22°C until the end of the experiment. During the first week, 23 h of light was provided with a reduction to 20 h afterward. Free access to water and drug-free diets are provided for chicks. The ingredients and chemical composition of experimental diets are shown in Table 1. Five dietary treatments were as: control group (basal diet without supplementation); HY1: hydrolyzed yeast at level of 1.0 g/kg; HY2: hydrolyzed yeast at level of 2.0 g/kg; SPM1: SPM powder at level of 30 g/kg diet and SPM2: SPM powder at level of 60 g/kg diet. In HY diets, the HY was added as on top of the diets. The overall experimental period was divided into three phases: starter (1–10 days), grower (11–24 days), and finisher (25–42 days). Chickens and feed were weighed on day 42. Mortality was recorded and feed conversion ratio (FCR) was calculated with correction for mortalities.

Vaccination and serology

On days 10, 19, and 32, vaccination programme were done against Newcastle disease virus. On days 12 and 24, vaccination programme were done against infectious bursal disease virus (Gumboro, D78 as eye drop). On day 21, blood samples (4.0 ml) were collected in Venoject tubes containing EDTA from the wing veins of 10 birds per treatment. These blood samples were used for gene expression analyses, antibody titres, and counting total and differential white blood cells. Hemagglutination-inhibition test was used for the measurement of the amount of antibody titres against Newcastle virus disease as explained by Allan and Gough (1974). Before the statistical analysis, antibody titre values were transformed to log2(x). Enzyme-linked immunosorbent assay was applied for the measurement of antibody titres against infectious bursal disease viruses using antibody test kits (IDEXX Laboratories Inc., Westbrook, ME, USA).

Gene expression analysis

The blood samples of two chicks from each replicate were collected on day 21 of age. The collected samples were maintained in a liquid nitrogen tank until expression gene analyses. The gene expression of IL-2 was done using reverse transcription-polymerase chain reaction (PCR) technique. The mealion of messenger RNA was done based on a mealion kit (Vivantis Company, Malaysia). Then, cDNA was synthesized using enzyme (reverse transcriptase) based on a commercial kit (Vivantis Company, Malaysia). Real-time PCR technique was done using Power SYBR Green PCR Master Mix (Applied Biosystems, CA, USA). Beta-actin was applied as housekeeping gene.

Statistical analysis

Before statistical analysis, the normal distribution of the data was checked based on test of Kolmogorov–Smirnov. Statistical analyses were done with the general linear model procedure of SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC, USA) to determine if variables differed between groups. The Tukey test was used for comparing the treatment means. Probability values of less than 0.05 (P < 0.05) were considered significant.

Results and discussion

The results from SPM nutrients composition, AME, and AMEn are shown in Tables 2 and 3. As shown in Table 2, SPM is rich in protein and fat content with 77.3% crude protein and 12.5% fat content. The amino acid content in CP is comparable with soybean meal. SPM shows a good alternative protein source, providing the essential amino acids and acceptable AMEn for poultry compared to other animal protein sources such as meat meal and fish meal (FM).
The effect of HY and SPM supplementation on performance of broiler chickens is shown in Table 4. Feed intake, body weight gain, and FCR were affected by dietary treatments. SPM supplementation to diets improved daily gain compared to HY1 and control groups. The highest daily feed intake and daily gain was for birds in SPM and HY2 in comparison with HY1 and control groups. The higher feed intake in the SPM2 group resulted in higher daily gain compared to HY1 and control groups. There was no difference for daily gain of HY1 chickens compared to the control group. Dietary inclusion of 6 SPM (SPM2) resulted in higher feed intake compared to other groups. By increasing the level of HY and SPM, feed intake was increased significantly. The best FCR was for SPM2 and the worse was for the control group. Feeding HY2 improved FCR compared to control and HY1 groups.

The FCR improvement could be related to the supplementation of the HY products, which may improve gut health. The ability of yeast and its components to act as growth promoters associated with different mechanisms. For example, Saleh et al. (2011) reported that dietary supplementation of Aspergillus awamori and Aspergillus niger improves broilers weight gain and FCR. They proposed that WG and FCR improvements may be due to the increases in metabolisable energy and enzymes produced by Aspergillus. It has been reported that yeast favours the proliferation of beneficial microbes such as lactobacillus by using as substrates for

### Table 1. Ingredients and composition of the basal diets at starter period (1–10 days), growth period (11–24 days), and finisher period (25–42 days).

| Ingredients (%) | Starter | Grower | Finisher |
|-----------------|---------|--------|---------|
|                 | Control | 3% SPM | 6% SPM | Control | 3% SPM | 6% SPM | Control | 3% SPM | 6% SPM |
| Corn grain      | 53.5    | 57.8   | 61.9    | 59.7    | 63.7   | 67.8    | 64.6    | 68.6   | 73.0    |
| Soybean meal (45%) | 38.3   | 32.3   | 26.4    | 33.1    | 27.3   | 21.5    | 28.7    | 22.9   | 17.1    |
| Oil, vegetable  | 3.9     | 2.72   | 1.53    | 3.51    | 2.3    | 1.18    | 3.40    | 2.23   | 1.10    |
| CaCO3           | 0.66    | 0.79   | 0.93    | 0.80    | 0.93   | 1.06    | 0.74    | 0.88   | 1.01    |
| NaCl            | 0.25    | 0.25   | 0.25    | 0.28    | 0.28   | 0.28    | 0.30    | 0.30   | 0.30    |
| NaHCO3          | 0.19    | 0.19   | 0.19    | 0.14    | 0.14   | 0.14    | 0.07    | 0.07   | 0.07    |
| Vit. & Min. Mix | 0.50    | 0.50   | 0.50    | 0.50    | 0.50   | 0.50    | 0.500   | 0.500  | 0.500   |
| L-Lysine_HCl    | 0.20    | 0.20   | 0.19    | 0.20    | 0.19   | 0.17    | 0.16    | 0.15   | 0.13    |
| L-Threonine     | 0.04    | 0.05   | 0.06    | 0.04    | 0.04   | 0.05    | 0.03    | 0.04   | 0.04    |
| SPM             | 0       | 3.00   | 6.00    | 0       | 3.00   | 6.00    | 3.000   | 6.000  | 6.000   |

Nutrient composition (%)

Metabolizable energy (kcal/kg) 3000 3000 3000 3050 3050 3050 3100 3100 3100
Crude protein21.5 21.5 21.5 19.7 19.7 19.7 18.0 18.0 18.0
Calcium 0.90 0.90 0.90 0.84 0.84 0.84 0.76 0.76 0.76
Available phosphorus 0.45 0.45 0.45 0.42 0.42 0.42 0.38 0.38 0.38
Sodium 0.16 0.16 0.16 0.16 0.16 0.16 0.16 0.16 0.16
Lysine 1.32 1.32 1.32 1.19 1.19 1.19 1.05 1.05 1.05
Methionine 0.65 0.66 0.67 0.58 0.58 0.58 0.53 0.54 0.55
Methionine + cysteine 0.98 0.98 0.98 0.89 0.89 0.89 0.82 0.82 0.82
Threonine 0.86 0.86 0.86 0.81 0.81 0.78 0.71 0.71 0.71

Note: SPM, spleen meal.

### Table 2. Nutrient composition (%) and AME and AMEn of SPM.

| Nutrient         | Dry matter | AME (kcal/kg) | AMEn (kcal/kg) | CP | Ether extract | Ca | P | Zn | Fe | Cu |
|------------------|------------|---------------|----------------|----|--------------|----|----|----|----|----|
|                  | As fed (%) | 3496          | 3296           | 77.3 | 12.5 | 0.038 | 1.09 | 0.23 | 0.0012 | 0.0078 |

Note: SPM, spleen meal.

### Table 3. Amino acid composition of the SPM.

| Amino acid       | Standardized to a dry matter content of 88% | AA (%) in CP | Content (%) as fed |
|------------------|-------------------------------------------|--------------|-------------------|
| Lysine           | 4.91                                      | 0.86         | 5.3               |
| Methionine       | 1.33                                      | 1.86         | 1.44              |
| Threonine        | 2.68                                      | 3.74         | 2.89              |
| Leucine          | 5.79                                      | 8.08         | 6.25              |
| Iso-leucine      | 2.31                                      | 3.23         | 2.50              |
| Valine           | 3.95                                      | 5.52         | 4.27              |
| Glutamic acid    | 8.30                                      | 11.60        | 8.96              |
| Met + Cys        | 2.01                                      | 2.81         | 2.17              |
| Arginine         | 3.84                                      | 5.36         | 4.15              |
| Histidine        | 1.87                                      | 2.61         | 2.02              |
| Phenylalanine    | 3.03                                      | 4.23         | 3.27              |
| Glycine          | 4.49                                      | 6.27         | 4.85              |
| Serine           | 2.68                                      | 3.74         | 2.89              |
| Proline          | 3.33                                      | 4.65         | 3.60              |
| Alanine          | 4.72                                      | 6.59         | 5.09              |
| Aspartic acid    | 5.70                                      | 7.95         | 6.15              |

Note: SPM, spleen meal.

### Table 4. Effects of HY and SPM on growth performance of broilers 0–42 days.

| Treatment | Daily feed intake (g) | Daily gain (g) | FCR |
|-----------|-----------------------|----------------|-----|
| Control   | 108.37b               | 54.36b         | 1.99a |
| HY1       | 105.97c               | 55.33c         | 1.92b |
| HY2       | 100.76b               | 57.93b         | 1.90c |
| SPM1      | 109.70b               | 58.78b         | 1.86d |
| SPM2      | 113.47a               | 61.50a         | 1.85f |
| SEM       | 0.56                  | 0.34           | 0.015 |
| P value   | <0.0001               | <0.0001        | <0.0003 |

Note: Columns with different superscript letters are significantly different (P < 0.05); SPM, spleen meal; HY, hydrolyzed yeast.
these microbes in the gut (Pan and Yu 2014). HYs have a cell wall comprising mainly β-glucans and mannanoligosaccharides; these components appear to affect the immune system and are able to prevent pathogenic bacterial colonization in the gastrointestinal tract. HYs also have cell content with free nucleotides that affect animal intestinal health, thereby increasing growth and positively influencing the bacterial flora in monogastric animals (Araujo et al. 2018).

There was no significant difference for FCR between HY1 and SPM1. In the literature, there was no study concerning the effect of HY and SPM on performance of animals. The improved growth performance resulted by SPM supplementation may be related to improved feed intake in SPM groups. Karimi (2016) observed improved performance for birds fed FM diets (0–5%) as compared to birds fed SBM diets. The improved ADFI and BWG observed for the birds fed SPM shows that protein source can support broiler growth performance. In a similar way, researchers have reported that the use of FM in broiler diets resulted in improved ADFI and BWG for birds fed such diets (Karimi 2006; Cho and Kim 2011). The researchers attributed their findings to the nutritional profile of FM, which impacted its palatability. The improved FCR in SPM groups also suggests that there may have been an improvement in digestibility of the amino acids in the diets which may have been due to changes in the intestinal physiology of birds. Beski et al. (2015) reported that the high digestibility of FM (>90%) improves FCR and support faster growth. Also it is suggested that in animal source are creatine and guanidine acetic acid (GAA) sources which maybe not enough in plant base diets. Dietary addition of GAA resulted in FCR improvement in broilers (Mousavi et al. 2013).

The highest total white blood cells were for SPM groups (Table 5). HY had no effect on white blood cells, but influenced on heterophil count. The lowest count of heterophil was for HY1 and HY2 groups. The highest platelet was for SE2 and the lowest counts were for HY1 and HY2.

Table 6 shows the geometric means of antibody titres against Newcastle virus and infectious bursal virus. There was difference among treatments for antibody titre against infectious bursal virus, but not against Newcastle virus. Antibody titre against infectious bursal virus was the lowest in control and HY1 groups. HY2, SPM1, and SPM2 had higher titre than two other groups.

The effect of SPM on antibody titre may be related to its Tuftsin as it is an immune modulating peptide. In the animal model, it was reported that Tuftsin could increase the counts

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**Table 5.** Total and differential counts of white blood cells in broilers.

| Item     | Control | HY 1 | HY 2 | SE1 | SE2 | SEM  | P value |
|----------|---------|------|------|-----|-----|------|---------|
| White blood cells count (×10^4/µl) | 9.44^b | 8.59^b | 8.46^b | 9.95^b | 10.59^a | 0.600 | 0.040   |
| Heterophils (%) | 3.49^a | 2.26^c | 2.18^b | 2.80^b | 2.75^b | 0.008 | 0.008   |
| Lymphocytes (%) | 6.51 | 6.17 | 6.12 | 6.38 | 6.91 | 0.936 | 0.030   |
| Monocytes (%) | 0.032^b | 0.011^c | 0.013^c | 0.028^b | 0.040^b | 0.003 | 0.007   |
| Platelets (%) | 321^b | 194^c | 168^c | 255^c | 357^c | 0.360 | 0.320   |
| RBC | 5.04 | 4.25 | 4.05 | 4.97 | 5.05 | 0.100 |         |

Note: Columns with different superscripts are significantly different (P < 0.05).

**Table 6.** Effects of HY and SPM on antibody titres (log 2) against NDV and IBD.

| Item     | Control | HY1 | HY2 | SPM1 | SPM2 | SEM  | P value |
|----------|---------|-----|-----|------|------|------|---------|
| NDV      | 4.00   | 4.66 | 4.40 | 4.13 | 4.90 | 0.476 | 0.100   |
| IBD      | 409^b | 414^b | 421^a | 425^a | 443^a | 15.7  | 0.030   |

Notes: Column with different superscripts are significantly different (P < 0.05). SPM, spleen meal; HY, hydrolyzed yeast.

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**Figure 1.** Relative gene expression of IL-2 in broilers fed control diet, SPM, and HY.
of monocytic cells (Liu et al. 2012; Gao et al. 2015). In a study, Saravanabava et al. (2005) evaluated the effect of Tuftsin on immune response of birds vaccinated with Newcastle virus vaccine. The results revealed that Tuftsin produced significant increase in the serum antibody titres in birds vaccinated along with Tuftsin and they confirmed the immunostimulatory effect of Tuftsin in poultry. Increases in antibody titre in HY2 may be related to two components in this product, MOS and B-glucan (Kogan and Kocher 2007; Moon et al. 2016). As shown in Table 5, HY increased the count of white blood cells and these changes could also influence the level of antibody titre. The immune stimulatory properties of β-glucan (in various forms) have been reported in a variety of species (Chae et al. 2006; Moon et al. 2016). In agreement to our results, Chen et al. (2006) and Cox et al. (2010) reported that β-glucan had immune enhancing effect in challenged chickens.

The effect of treatments on interleukin-2 gene expression is presented in Figure 1. The highest gene expression found in broilers received SPM2 and the lowest was for those received control and HY. In animal models, it was reported that SPM could enhance macrophage activity and immunoglobulin production (Liu et al. 2012; Gao et al. 2015). In the immune system, IL-2 is a type of cytokine signalling molecule that regulates the activities of white blood cells responsible for antibody production. Dietary SPM supplementation increased IL production and finally affected on antibody titres against infectious bursal virus.

It was demonstrated that yeast and yeast derivatives involved in the synthesis of the pro-inflammatory cytokine such as TNF-α in macrophages also influence on the release stimulation of cytokines (Majtán et al. 2005). They are involved in the release of other cytokines such as IL-1, IL-2, and IL-6 (Gantner et al. 2003; Brown 2006).

The polysaccharide β-glucan in HY is classified as a huge biological response modifier (Bohn and Bemiller 1995). Also it was shown that β-glucan could increase the macrophages and neutrophils functionality (Williams et al. 1996). The exact molecular mechanisms that mediate the immunomodulatory activities of β-glucan are not clear.

Conclusion

It was concluded that inclusion of SPM and HY in the diet improved broiler performance and increased the immune response and antibody titre in broiler chicks. The effect of SPM was higher than HY as immune enhancer.

Disclosure statement

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

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