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Research article

Effect of soybean waste fermented by *Saccharomyces cerevisiae* on growth performance, hematological parameters, liver enzyme functions, and immunity of Vietnamese native chickens

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Abstract

Fermented soybean waste (FSW), produced by fermentation by *Saccharomyces cerevisiae*, has emerged as a promising feed ingredient for poultry because of its potential to improve growth performance, health, and immune responses. This study aimed to evaluate the effects of dietary supplementation with FSW at various levels on growth performance, serum biochemistry, liver enzyme activity, and antibody titers against Newcastle disease in Vietnamese native chickens (Noi chickens). A total of 128 native chickens were assigned to four dietary treatments: control (0% FSW), 10% FSW (S1), 20% FSW (S2), and 30% FSW (S3). Each treatment was replicated four times. The experiment was conducted in mice between 5 and 12 weeks of age. Chickens in the S3 group showed higher body weights and average daily weight gain during weeks 5–9, along with an improved feed conversion ratio ($p < 0.05$). Serum biochemical and liver enzyme profiles remained within physiological ranges and did not differ significantly among treatments, indicating no adverse metabolic or hepatic effects. Newcastle disease virus antibody titers were elevated in S3 at 28 and 42 days of age ($p < 0.05$), suggesting a relatively enhanced early and sustained immune response compared to the control. By day 56, the antibody titers were no longer different among the groups ($p > 0.05$), suggesting a transient immunomodulatory effect. In conclusion, dietary inclusion of FSW, particularly at 30%, improved growth performance and immune responses in native chickens without negatively affecting liver or metabolic health.

Keywords: Fermented feed, Growth, Hematological, Immunity

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INTRODUCTION

The increase in native poultry plays an important role in Vietnam's agriculture, particularly in rural areas. Chicken breeds native to Vietnam, especially the Noi breed, are favored by consumers because of advantages such as good resistance, adaptability to natural conditions, and high meat quality (Khoa et al., 2019; Linh et al., 2020). Moreover, native chickens are particularly important in functional feed research because of their slow growth rate and longer rearing periods, which make feed costs a more significant component of production expenses (Linh et al., 2020). However, raising native chickens is associated with many challenges owing to high feed costs, low farming productivity, and disease pressure. A large portion of the costs of poultry farming comes from feed, primarily industrial feed (Linh et al., 2022), making feed optimization a crucial factor for improving economic efficiency.

Recently, the use of agricultural and food industry byproducts in livestock farming has become popular to reduce costs and minimize environmental pollution (Ababor et al., 2023). Byproducts such as cassava pomace (Aroh et al., 2024) as well as soy (Azmi et al., 2023) help utilize available resources and contribute to improving the gut microbiota and animal health through fermentation (Predescu et al., 2024). Fermented soybean waste (FSW) has been evaluated as a nutritious and potential raw material for the poultry industry. FSW, a byproduct of soymilk and tofu production, is widely used in poultry farming because of its high protein content. However, anti-nutritional factors such as oligosaccharides, trypsin inhibitors, and phytic acid in soybean meal frequently reduce nutrient absorption by poultry (Suprayogi et al., 2022). To overcome this limitation, FSW has been treated using various methods, including fermentation with the yeast *Saccharomyces cerevisiae*, which helps increase its nutritional value and improve growth and development in poultry farming (Sugiharto and Ranjitkar, 2019). The fermentation process helps break down anti-nutritional factors and produces many beneficial compounds, including bioactive peptides, short- chain fatty acids, digestive enzymes (protease and amylase), B vitamins, and probiotics that can support the balance of the gut microbiota (Sugiharto et al., 2021). Beneficial bacteria produced during fermentation, such as *Lactobacillus* spp., *Bifidobacterium* spp., and *Enterococcus* spp., when ingested by poultry, help enhance the stability of the intestinal microbiota, inhibit the growth of harmful bacteria, and improve food digestion efficiency (Sadh et al., 2018; Kumar et al., 2025). Thus, poultry can optimally absorb nutrients, which promotes growth, increases productivity, and reduces the risk of gastrointestinal diseases (Hong et al., 2004; Feng et al., 2007). FSW with *S. cerevisiae* has been proven to be safe for poultry health (Chachaj et al., 2019; Obianwuna et al., 2024). The addition of this product to the diet alters blood biochemical indices such as glucose, cholesterol, aspartate aminotransferase (AST), and alkaline phosphatase (ALP) enzymes—factors which reflect liver function (Muniyappan et al., 2023). Fermented soybean meal also improves the natural immunity of poultry by stimulating antibody production and enhancing immune cell activity (Chachaj et al., 2019). The addition of fermented soybean meal to poultry diets improves digestive capacity and stimulates the development of immune organs, especially in breeding poultry (Jazi et al., 2018; Chachaj et al., 2019; Lu et al., 2019). However, supplementation of fermented soybean meal has not produced significant effects on blood biochemical parameters or immunity in broiler chickens, and there have been no recorded negative impacts of FSW on poultry health. Particularly, the effects of FSW are unclear.

Although FSW has shown potential for improving the health and productivity of poultry, research on the detailed effects of this product on the growth performance, meat quality, health, and immune system of local chickens when added to their diet remains limited. Despite its promising nutritional and functional benefits, concerns may arise regarding the potential toxicity of FSW when included at high dietary levels. Previous studies have validated its safety. Chachaj et al. (2019) reported no adverse effects on the liver or immune parameters in broilers fed fermented soybean meal. Saleh et al. (2021) also observed no alterations in the liver enzyme profiles when fermented feed ingredients were used. Therefore, this study was conducted to evaluate the effects of adding FSW fermented by *S. cerevisiae* to the diet on the growth, blood parameters, and immune capacity of native chickens.

MATERIALS AND METHODS

Location and experimental period

This study was conducted at the Animal Experimental Farm of Tra Vinh University, located in Tra Vinh City, Vietnam, from September to December 2024. All procedures involving animals followed ethical standards established by the Science and Technology Council and were approved under decision number 137/2022/HĐ. HĐKH&ĐT-ĐHTV.

Preparation of soybean waste anaerobic fermentation

Soybean waste was fermented as follows: 1 kg of soybean meal was mixed with 0.3% *S. cerevisiae* and 0.3% molasses. Before mixing, a quantity of warm water (approximately 37 °C), accounting for 20% of the soybean waste's weight, was used to dissolve the yeast and molasses and was then incubated for approximately 3 hours to activate the fermentation process. The mixture was then evenly mixed with soybean residue and incubated in a sealed container for 12 hours. The fermented product met the requirements when it had a light, characteristic aroma without any mold or burnt smell. After fermentation, the mixture was immediately used as a chicken feed.

Feed preparation

Throughout the study period, all birds were maintained under consistent dietary and management conditions. The diet consisted of a formulated feed that satisfied the nutrient requirements of broiler chickens, as outlined by the NRC (1994). Before feeding, the ingredients were analyzed to confirm their nutritional composition. The experimental diets differed according to the inclusion rate of FSW: 0% (control), 10%, 20%, and 30%. Importantly, FSW was incorporated by replacing portions of multiple feed ingredients, including soybean meal, corn, rice bran, and fish meal, rather than adding them to a complete 100% formulation. This ensured that the formulation of each diet was 100%. To ensure consistency and comparability across treatments, all diets were carefully reformulated to be isonitrogenous (17%–20% crude protein, depending on the growth phase) and isoenergetic (from 3025–3100 kcal/kg metabolizable energy). The specific nutrient composition of each diet is shown in Tables 1 (weeks 1–4) and 2 (weeks 5–9).

Table 1 Feed formulation and proximate analysis of diet from 1-4 weeks old

Ingredient	Control	S1	S2	S3
Corn	22	17	13	8
Broken rice	17	17	17	17
Rice bran	32.1	29.7	26.1	24.3
Soybean meal	16	16	16	14
Fish meal	10.5	7.9	5.5	4.3
DCP	0.3	0.3	0.3	0.3
Stone	1.3	1.3	1.3	1.3
Lysine	0.1	0.1	0.1	0.1
Methionin	0.1	0.1	0.1	0.1
Mineral premix - vitamin	0.3	0.3	0.3	0.3
FSW	0	10	20	30
Salt	0.3	0.3	0.3	0.3
Total	100	100	100	100
CP	20	20	20	20
ME	3029	3021	3025.2	3026.7
Lys	1.17	1.12	1.08	1.04
Met	0.58	0.58	0.58	0.60
Ca	1.05	1.05	1.07	1.13
P	0.69	0.69	0.68	0.70

Table 2 Feed formulation and proximate analysis of diet from 5-9 weeks old

Ingredient	Control	S1	S2	S3
Corn	32	28.5	27	26
Broken rice	17	18	16	14
Rice bran	27.6	22.1	18	12.9
Soybean meal	16	14	11.6	9.7
Fish meal	5	5	5	5
DCP	0.3	0.3	0.3	0.3
Stone	1.3	1.3	1.3	1.3
Lysine	0.1	0.1	0.1	0.1
Methionin	0.1	0.1	0.1	0.1
Mineral premix - vitamin	0.3	0.3	0.3	0.3
FSW	0	10	20	30
Salt	0.3	0.3	0.3	0.3
Total	100	100	100	100
CP	17	17	17	17
ME	3099	3099	3096	3097
Lys	0.97	0.96	0.95	0.95
Met	0.51	0.53	0.53	0.54
Ca	0.86	0.93	1.01	1.11
P	0.58	0.55	0.53	0.50

Bird management

The study employed a completely randomized design with four dietary treatments and four replicates reflecting FSW inclusion levels. The control group was fed the standard diet without FSW, whereas the other groups were supplemented with 10% (S1), 20% (S2), or 30% (S3) FSW. A total of 128 Noi chickens (64 males and 64 females) were used and divided into experimental units of eight birds with similar body weights per replicate. Each chicken was housed in an individual cage (100 × 100 × 60 cm) equipped with a wire mesh floor, passive ventilation, and exposed to natural lighting with an average daytime temperature ranging from 28 °C to 32 °C. Routine vaccinations were administered, including those for avian influenza, fowl pox, gumboro (first and second doses), and Newcastle disease virus (NDV, LaSota strain, Navetco Company, Vietnam) (first and booster doses), using oral, ocular, wing web, and intramuscular methods. The birds were fed twice daily at 6:30 AM and 5:00 PM. Feed leftovers were weighed at 7:00 AM the following morning to monitor feed intake. Clean drinking water was available at all times through the farm water systems. Daily cleaning of feeders and drinkers, along with regular cooperative sanitation, was performed to maintain biosecurity and limit disease risk.

Growth performance

Growth performance of the chickens was assessed by measuring body weight (BW), feed intake (FI), and feed conversion ratios (FCR). Body weights were recorded at the beginning of the trial (5 weeks of age) and subsequently every two weeks until 12 weeks of age. Average daily gain (ADG) was determined by dividing the weight gained over the trial period by the total number of feeding days. FI was calculated by subtracting feed refused from the amount of feed offered. The feed conversion ratio was then computed by dividing the total feed consumed by the total weight gain to provide an index of feed efficiency.

Blood profiles

At the end of the experiment (when chickens were 56 days old), two chickens were randomly selected from each experimental unit to collect blood samples for analysis of serum biochemical parameters and liver function assessment. Approximately 2 mL of blood was drawn from the wing vein using a 5 mL sterile syringe with a 23 G needle. Blood samples were placed in tubes containing EDTA for hematological and liver function tests. Immediately after collection, the blood tubes were stored in cold bags and transported to the laboratory within 24 hours. Subsequently, blood samples were centrifuged at 3,000 rpm for 15 minutes to separate the serum. The serum was used to analyze biochemical indicators using an SMT-120VP automatic analyzer (Chengdu Seamaty Technology Co., Ltd., China). The measured serum parameters included total protein, albumin, globulin, glucose, total cholesterol, triglyceride, low-density lipoprotein (LDL-c), and high-density lipoprotein (HDL-c) levels. To assess liver function, alanine aminotransferase (ALT), AST, ALP, and gamma-glutamyl transferase (GGT) levels were analyzed. All results are expressed as mg/dL for metabolic parameters and U/L for liver enzymes.

Antibody titer

Before administering the NDV vaccine, the chickens were tested for antibody titers to ensure that all birds tested negative for NDV. Blood samples were collected from the wing veins of individuals at 28, 42, and 56 days of age in accordance with the QCVN 01-83:2011/BNNPTNT regulations. Each sample was obtained from 2 mL of blood using a pre-labeled sterile syringe. After collection, the syringes were placed horizontally in a sample container, allowing them to clot naturally at room temperature for 1 to 2 hours.

Blood samples were stored in a cold box at a temperature of 2–8 °C and transported to the analysis center. The serum obtained from the samples was used to determine the NDV antibody titer by ELISA using a commercial kit (Idvet kit; Innovative Diagnostics, Grabels, France). Post-vaccination titer values ≥993 were considered to achieve a level of active immune protection.

Data analysis

All raw data were compiled and preliminarily processed using Microsoft Excel 365 software (Microsoft Corp., Redmond, WA, USA). Statistical analysis was performed using a generalized linear model with Minitab version 2016 software (Minitab LLC, State College, PA, USA). Illustrative charts were constructed using GraphPad Prism, version 9 (GraphPad Software, La Jolla, CA, USA). Tukey's test was used to compare the means between treatments, and statistical significance is reported at a threshold of $p < 0.05$.

RESULTS

Growth performance of native chickens from 1–9 weeks of age

The addition of FSW had a positive effect on several growth indicators and feed efficiency of chickens during the 5–9 week period. Specifically, BW at week 9 and ADG during this period increased in the supplemented groups, especially in the S3 group, compared with the control ($p < 0.05$). Simultaneously, FCR in the S3 group decreased, reflecting a considerable improvement in feed efficiency ($p < 0.05$). Although no statistical differences were observed in BW, FI, and FCR during the 1–4 week period, an improvement trend was still observed in the supplemented groups compared with the control group. Overall, the results indicate that the use of FSW, especially at higher supplementation levels, can improve growth performance and feed efficiency. The overall FCR was naturally higher during weeks 5–9 than in the early phase owing to increased maintenance requirements and slower growth efficiency in older birds. However, dietary supplementation with 20%–30% FSW improved FCR during this period, suggesting enhanced nutrient utilization and better adaptation to dietary changes in these groups.

Table 3 Growth performance of chickens under FSW diets

Criterion	Groups				SEM	*p*
	Control	S1	S2	S3		
Body weight, g/bird						
Initial BW	28.87	27.83	27.87	28.06	0.341	0.152
4 weeks old	206.4	234.7	210.3	229.7	9.158	0.113
9 weeks old	680.1^{b}	713.6^{ab}	745.1^{ab}	787.5^{a}	19.33	0.008
ADG, g/bird/day						
1-4 weeks old	6.342	7.387	6.515	7.200	0.321	0.094
5-9 weeks old	13.53^{b}	13.68^{b}	15.28^{a}	15.94^{a}	0.266	0.001
FI, g/bird/day						
1-4 weeks old	12.35	12.75	12.55	12.22	0.250	0.483
5-9 weeks old	42.50^{a}	41.72^{ab}	41.06^{b}	40.62^{b}	0.339	0.007
FCR						
1-4 weeks old	1,960	1,730	1,952	1,719	0,092	0.140
5-9 weeks old	3.463^{ab}	3.528^{a}	3.034^{ab}	2.98^{b}	0.131	0.015

Different superscript letters (a, b, c) in the same row shows statsitically significant differences ($p < 0.05$).

Hematological parameters

These serum biochemical parameters provide insight into the metabolic status and organ health of birds. The fact that no significant changes were observed suggests that dietary FSW does not cause metabolic stress or liver dysfunction. None of the serum biochemical indicators (Figure 1), including total protein (A), glucose (B), cholesterol (C), triglycerides (D), albumin (E), and globulin (F), were different between the experimental groups ($p > 0.05$). These results indicate that supplementation does not adversely affect the basic metabolism and physiological conditions of poultry.

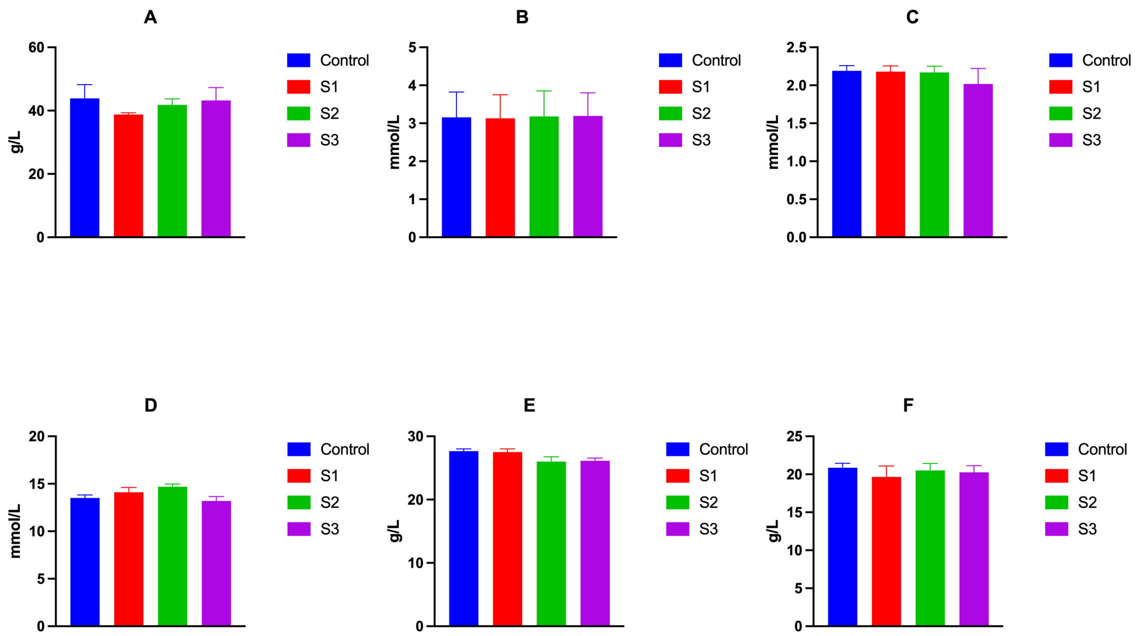

Figure 1 Effect of FSW by *S. cerevisiae* on blood profiles of chickens at 9 weeks of age. A: total protein; B: total cholesterol; C: triglycerides; D: glucose; E: globulin; F: Albumin. Figure 1A, 1B, 1C, 1D, 1E, and 1F shows no significant differences between treatments ($p > 0.05$).

Liver enzyme function

Among the liver enzyme indices analyzed (Figure 2), ALT activity (Figure 2B) in the S3 group tended to be higher than that in the control group; however, the difference was not ($p > 0.05$). Other enzymes, such as AST (A), ALP (C), and GGT (D), were not significantly different between the groups. This indicates that the treatments did not negatively affect liver function in the animals.

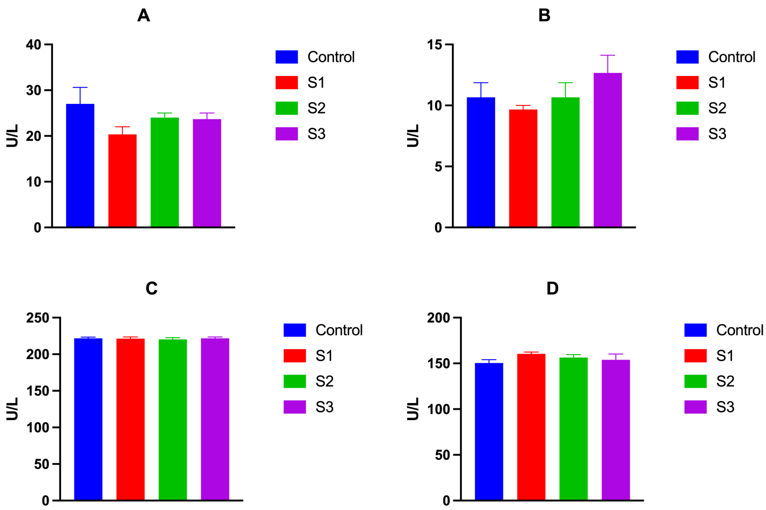

Figure 2 Effect of FSW by *S. cerevisiae* on liver enzyme function of native chickens in the experiment. A: GGT; B: ALT; C: AST; D: ALP. Figure 2A, 2B, 2C and 2D shows no significant differences between treatments ($p > 0.05$).

Antibody titer

Figure 3 shows antibody titers against NDV supplemented with FSW. At 28 days, the antibody titer (Figure 3A) of the S3 group was higher than that of the control ($p < 0.05$), reflecting a good early immune stimulation capability. Similarly, on day 42 (Figure 3B), the S3 group maintained higher antibody titers than the other groups ($p < 0.05$), indicating the prolonged efficacy of this regimen. The significantly higher ND antibody titers in the S3 group on days 28 and 42 indicated an enhanced humoral immune response. This suggests that high levels of dietary FSW may support early immune activation and antibody production following vaccination. However, by day 56 (Figure 3C), there was no difference between the groups ($p > 0.05$), indicating that the immune response was not affected by FSW supplementation. However, by day 56, the titers showed no significant differences, possibly because of waning immunity.

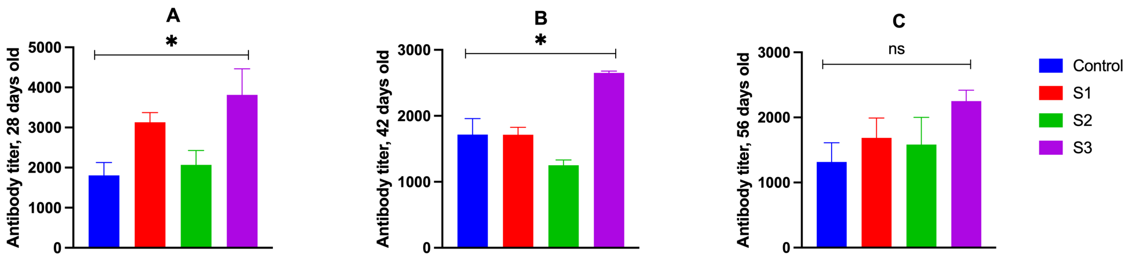

Figure 3 Effect of FSW fermented by *S. cerevisiae* on antibody titer against NDV vaccination. A: antibody titer at 28 days old; B: antibody titer at 42 days old; C: antibody titer at 56 days old. Figure 3A and 3B shows statistically significant differences between treatments ($p < 0.05$) whereas no effect is indicated in Figure 3C ($p > 0.05$). Note: * indicates statistical significance; ns: not significant.

DISCUSSION

The improvement in the growth rate of chickens supplemented with 30% FSW may be explained by various biological mechanisms related to digestion and nutrient absorption. The fermentation process helps break down anti-nutritional factors (Sugiharto and Ranjitkar, 2019) such as trypsin inhibitors, oligosaccharides, and phytate (Suprayogi et al., 2022) which occur in untreated soybean meal. Consequently, their ability to digest proteins and absorb minerals is significantly improved (Hong et al., 2004; Feng et al., 2007). In addition to eliminating adverse factors, the fermentation process produces many biologically active compounds such as small peptides, free amino acids, and organic acids. These compounds enhance gut health and stimulate the activity of digestive enzymes, helping chickens utilize feed more efficiently (Muniyappan et al., 2023). Irawan et al. (2022) also noted an increase in the height of intestinal villi and the depth of crypts in the small intestine of chickens supplemented with fermented soybean meal, which increased the surface area for nutrient absorption. Similarly, Li et al. (2020) showed that a diet containing fermented plant proteins improved intestinal morphology and increased the expression of nutrient transport genes, thereby enhancing metabolic efficiency. Changes in the structure of the gut microbiota also play an important role. The addition of FSW helps increase the density of beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, while inhibiting pathogenic microorganisms, contributing to the stabilization of the microbiome and reducing the risk of intestinal inflammation, as indicated in a study using fermented soybean meal (Jazi et al., 2018).

The lack of significant changes in serum biochemical parameters between the treatments indicated that the use of FSW, even at high levels (30%), did not disrupt the basic metabolism of the chicken body. Parameters such as total protein, albumin, and globulin directly reflect the status of protein synthesis and immune balance; therefore, the stability of these indicators suggests that liver and immune functions are not adversely affected. Similarly, blood glucose and lipid concentrations reflect energy metabolism; if there is a metabolic disorder, these indicators frequently change significantly. This result is consistent with those of previous studies (Sugiharto et al., 2016; Sugiharto et al., 2018; Sugiharto and Ranjitkar, 2019) , where the use of fermented soybean meal in chicken diets did not significantly affect serum indices but also supported microbial balance and enhanced resistance (Qui and Linh, 2023; Qui et al., 2024). Regarding liver enzymes, indicators such as ALT, AST, ALP, and GGT, are sensitive biochemical markers of liver damage and functional stress. ALT and AST levels primarily reflect the integrity of the liver cells, whereas ALP and GGT levels are related to phosphate metabolism and bile function. The stability of these enzymes indicates that fermented soybean meal does not cause liver toxicity or exert abnormal metabolic pressure on the hepatobiliary system. Although ALT in the S3 group increased slightly, it remained within the normal physiological range for poultry and was not accompanied by changes in other enzymes, thus providing insufficient evidence to confirm a negative impact. This is similar to the observations of Saleh et al. (2021) who found that the use of fermented ingredients in the diet did not alter liver enzyme activity in broiler chickens.

The relatively higher antibody titers observed in the FSW-supplemented group, especially at 28 and 42 days of age, suggest that this ingredient may support immune responsiveness. Although the ELISA-derived titer value in the S3 group on day 28 was notably high (approximately 4000), it should be interpreted with caution, as only a booster dose of the vaccine was used. Thus, these results should be interpreted as a relative enhancement of the immune response rather than an unusually elevated absolute titer. The mechanism may stem from the fermentation process, which helps break down the anti-nutritional factors present in raw soybean meal, while also increasing free amino acids and other bioactive compounds (such as metabolized isoflavones and organic acids) (Azrinnahar et al., 2021; Soren et al., 2024). These compounds can stimulate immune cells such as B lymphocytes and macrophages to function more effectively, thereby promoting antibody production after vaccination (Amer et al., 2023). In addition, fermentation with *S. cerevisiae* increases the content of certain organic acids such as lactic acid and B vitamins, contributing to favorable conditions for the development of beneficial microbiota in the intestines (Azrinnahar et al., 2021; Soren et al., 2024). A stable microbiome environment helps improve gut health and enhance the function of the lymphoid tissues associated with the intestinal mucosa, thereby supporting immune responses. In addition, substances such as lactic acid and beta-glucan found in fermented corn support the digestive process and enhance the immune system of chickens (Azrinnahar et al., 2021; Qui and Linh, 2023). According to Zhang et al. (2022), fermented soybean products help activate immune-related genes and significantly improve IgG and IgM levels in poultry serum. Additionally, fermented products can improve the intestinal microbiota, thereby supporting the mucosal immune system, which plays a crucial role in systemic immune responses. This aligns with the assertion of Dai et al. (2020) who suggested that plant proteins, after fermentation, can act as prebiotics, enhance innate immunity, and achieve higher antibody titers post-vaccination (Khonyoung et al., 2025). Similarly, Sugiharto et al. (2016) noted the early immune-stimulating effects of fermented ingredients in broiler chicken diets. However, by the age of 56 days, when the immune response had stabilized or entered the natural declining phase after active immunization, the differences between treatments were no longer significant. This is the normal physiological progression of the immune system after vaccination and reflects that the addition of FSW does not inhibit or hinder the subsequent immune response.

CONCLUSION

The addition of FSW to the diet of indigenous chickens had positive effects on growth, feed efficiency, and immune response without adversely affecting serum biochemical indices or liver function. In particular, the 30% supplementation level showed the most significant effects, with faster weight gain, lower FCR, and higher ND vaccine antibody titers than those in the control group. These results indicate that FSW is a potential ingredient in poultry nutrition and can be used as a functional component in feed to enhance the performance of indigenous chicken in a sustainable and biosecure manner. Besides, this study was limited to short-term evaluation (up to 12 weeks), focusing on growth, blood parameters, and immune response. Long-term impacts of FSW on production traits, carcass quality, and sustained immunity in native chickens should be examined in future studies.

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AUTHOR CONTRIBUTIONS

Nguyen Hoang Qui: Conceptualization; Data Curation; Formal Analysis; Investigation; Methodology; Supervision; Validation; Writing – Original Draft Preparation; Writing – Review & Editing (lead). Nguyen Thuy Linh: Conceptualization; Data Curation; Formal Analysis; Investigation; Methodology (lead); Supervision; Validation; Writing – Original Draft Preparation; Writing – Review & Editing (supporting).

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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