

Original article

Contents lists available at ScienceDirect

# Saudi Journal of Biological Sciences



journal homepage: www.sciencedirect.com

# Sprouted rough rice as an alternative to corn for growth, health performance and meat quality of broilers



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#### ARTICLE INFO

Keywords:

β-Glucan

Fatty acids

Growth

Germinated rice

Hepatic enzymes

Blood

#### ABSTRACT

Sprouted rough rice (SR) is not commly used for poultry diets but it could be a potential replacement ingredient to improve nutritional value of feed. This study aimed to evaluate the growth performance, carcass quality, blood lipids, hepatic enzymes, and fatty acid profiles of broiler meat when corn was replaced with sprouted rough rice in the feed. A completely randomized design was used with four groups and six replicate groups with 10 birds per replicate. The 4 treatment groups were 0, 15, 40 and 45 % sprouted-rice-replacement of corn. Growth performance and carcass quality were highest when corn was replaced with 45 % SR (p < 0.05). In particular, live weight and body weight gain increased linearly with increasing SR inclusion, whereas feed conversion ratio and feed intake linearly decreased with increasing SR (p < 0.05). Carcass, thigh, and breast weights showed the same trend of increase (p < 0.05). In addition, the pH of the thigh meat was significantly higher in diet with 45 % SR than in the other treatment groups. With increasing SR content, the weight of immune organs linearly increased, particularly the thymus and spleen (p < 0.05). Additionally, the fatty acids examined including saturated fatty acids, unsaturated fatty acid, transfat were mostly highest when 30 or 45 % of corn was replaced with SR and linearly increased from 0 to 45 % of SR. There were no significant differences of linear relationships with cooking loss, or blood lipid and hepatic enzyme profiles between treatments (p > 0.05). Replacing 45 % of corn in a broiler diet with SR could improve the growth and meat paramenters of broilers.

# 1. Introduction

Poultry farming in Vietnam is an important agricultural sector, contributing to ensuring social and food security and increasing the income of farmers. With the increasing human demand, poultry production should focus on the productivity and quality of poultry products, including meat. In addition to their meat being a source of protein for consumers (Linh et al., 2020), Noi broilers in Vietnam are adaptable to the local environment (Vo et al., 2019).

To improve growth performance and health without reducing economic efficiency, scientists have studied supplementation with probiotics, acidifiers, prebiotics, and various medicinal plants and/or herbs (Roy et al., 2015). Further, replacing expensive ingredients, such as corn, with alternative feedstuffs has been considered to reduce total feed costs (Linh et al., 2020; Saleh and Alzawqari, 2021). As similar to sprouted rough rice (SR), hydroponic wheat sprouts are natural and safe feed additives with the potential to enhance the production of highquality and safe chicken meat (Sultana et al., 2022). Germination leads to significant alterations in the physical and chemical makeup of wheat, which, when consumed by pigs and fowl, has been shown to not have any detrimental effects (Johnson and Taverner, 2019). Hydrolytic enzymes and other biological components become active during germination, breaking down starch, amino acids and polysaccharides to produce useful components (Oh et al., 2010). Sprouting grains can also decrease the presence of anti-nutritional compounds, such as phytic acid and tannins, making them easier to digest and utilize by the animal

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#### https://doi.org/10.1016/j.sjbs.2024.104034

Received 20 April 2024; Received in revised form 26 May 2024; Accepted 28 May 2024

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Abbreviations: ANOVA, analysis of variance; AOAC, Association of Official Analytical Chemists; NRC, National Research Council; BWG, body weight gain; CF, crude fiber; CP, crude protein; DM, dry matter; DCP, dicalcium phosphate; EDTA, ethylenediaminetetraacetic acid; EE, ether extract; ELISA, enzyme-linked immunosorbent assay; FA, fatty acid; FCR, feed conversion ratio; FI, feed intake; SR, sprouted rough rice; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; ME, metabolizable energy; MUFA, monounsaturated fatty acid; NFE, nitrogen-free extract; OM, organic matter; PUFA, poly-unsaturated fatty acid; SCFA, short-chain fatty acid; SEM, standard error of the mean; SFA, saturated fatty acid; MCT, medium-chain triglycerides; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

(Malama et al., 2020). Nutrient levels rise following grain germination, especially after 4–5 days (Agu et al., 2012). Germinated paddy rice contains 7.48 % crude protein (CP), 67.58 % nitrogen-free extract (NFE), 1.96 % ether extract (EE) and 12.55 % crude fibre (CF) on a dry matter (DM) basis. The gross energy of the germinated paddy rice is 3.77 kcal/g, a value similar to that of maize. Germinated paddy rice showed high levels of phytonutrients, particularly  $\gamma$ -amino butyric acid, which increased its antioxidant properties. Germinated paddy rice has been shown to be a viable alternative feed for chickens (Likittrakulwong et al., 2020). Further, sprouted rice contains β-glucan, substance that has been shown to improve poultry performance when included in the diet (Qui and Linh, 2023).

Sprouting, a methodology which improves the nutrient content of grains, has been applied in various studies on many plants. The addition of hydroponic wheat sprouts at 150 g/kg of feed notably enhanced the overall growth parameters of turkeys (Ali et al., 2019). In addition to growth performance, sprouted seeds have been shown to positively impact animal health. In particular, feeding sprouted rice has been shown to improve poultry health as proven through the immune organs and blood profiles. A previous study of (Sultana et al., 2022) showed that incorporating 50 g/kg of wheat straw into broiler diets positively affected blood lipid profiles by enhancing serum levels of cholesterol, triglycerides, and calcium, while also considerably boosting glucose and phosphorus concentrations. Sprouted rice can also affect antioxidant levels and immunological activity in broilers. As stated previously, the addition of sprouted rice to the diet was shown to enhance the growth parameters of broilers (Linh et al., 2020). This study used local broilers in Vietnam and showed that adding 75 mg of sprouted rough rice per kilogram of feed enhanced the growth parameters of broilers, including body weight and feed conversion ratio. A separate study found that the β-glucan concentration in fermented rice benefits the growth performance, carcass quality, fatty acid profiles, lipid profiles, and antibody titres of broilers (Qui and Linh, 2023). Further, the addition of 74 and 148 g/kg of dietary germinated paddy rice enhanced egg production in laying hens and altered protein profiles in eggs during stressful situations (Incharoen et al., 2021). Moreover, the presence of γ-amino butyric acid in SR has been shown to act as a growth stimulant for broilers, enhancing both production and reproduction (Incharoen et al., 2021).

Corn is not a common alternative ingredient in the diet. There are few studies on the function of sprouted rough rice as alternative for corn in feed, especially for broiler health and meat quality. The application of the therapeutic compounds presents in sprouted rough rice could be used to create a novel feed formulation for chicken production, potentially replacing corn in the diet. By this study, the effect of replacing corn with sprouted rough rice in the diet on growth parameters, carcass features, blood profiles, hepatic enzymes, and fatty acid profiles of chickens was investigated.

#### 2. Materials and methods

#### 2.1. Study site and animal ethics

This study began in October 2023 and concluded in January 2024 and was conducted at experimental farm of the Tra Vinh University in Tra Vinh Province, Vietnam. The animal care and use methods were accepted by the Research and Education Committee of Tra Vinh University under Decision No. 137/2022/HĐ.HĐKH&DT-DHTV.

#### 2.2. Germinated rice preparation

The rough rice was soaked in tap water at a temperature ranging from 25 to 30  $^{0}$ C for a duration of 48 h. The combination was stored at ambient temperature. Subsequently, the germinated rice was subjected to a process of drying out under the influence of sunshine for a duration of one day. Following germination, the sprouted rice was spread out in a tray for an additional 4 days in order to maximize the concentration of

nutrients and amino acids, as recommended by the study conducted by (Agu et al., 2012). A sprouted rice length of 2–3 cm was sufficient for grinding and mixing as feed. The germinated rice was pulverized to promote amalgamation with other constituents of the diet. Unsprouted rice was not chosen.

# 2.3. Diatery treatments and experimental design

In total, 240 broiler chickens (Noi crossbred chickens as one of local chickens in Vietnam) between 6 and 12 weeks of age were divided into four different treatments, with six replicate groups per treatment, using a completely randomised approach. Each replicate group contained ten birds evenly distributed by sex. During the initial five weeks, birds were provided consistent diet and environmental conditions to acclimatise to the experimental setup before any treatments were introduced. Birds were housed in cages which is 1 m higher than the floor. Diets were provided in mash form, and all birds had unrestricted access to both feed and water during the entire experiment. Once a week, the floor was cleaned and pen was disinfected, new husks containing Balasa bio-yeast (from Minh Tuan Factory, Ha Noi, Vietnam) were spread. No bird deaths occurred during the experiment. The investigation was carried out under natural light conditions with a natural day-light cycle at temperatures between 28 and 32 °C, with an average humidity of 60 % inside the experimental house. The experimental design included a control group receiving no sprouted rough rice (SR) in place of corn (SR0) and positive groups (SR1, SR2, and SR3) with varying amounts SR in place of corn: SR1 (15 % SR), SR2 (30 % SR), and SR3 (45 % SR). The replacements were fed for chickens from 6 to 12 weeks of age.

Feed materials were purchased from a local feed store in the Tra Vinh Province. Four treatments were developed to fulfil the needs of local birds aged 6–12 weeks based on the guidelines provided by the (NRC, 1994). Diet compositions are presented in Table 1 as analysed using the (AOAC, 1990) method. The chemical components of SR are detailed in Table 2.

# 2.4. Growth performance

The body weight (BW) and body weight gain (BWG) of the birds were determined weekly. The average weekly BWG per bird was determined

# Table 1

Feed formulation for the broilers from 7 to 12 weeks of age.

| Ingredients                | Replacem | nents |      |      |
|----------------------------|----------|-------|------|------|
|                            | SR0      | SR1   | SR2  | SR3  |
| Corn                       | 18       | 15    | 12   | 10   |
| Germinated rice            | 0        | 3.8   | 7.3  | 11   |
| Broken rice                | 22       | 22    | 22   | 22   |
| Rice bran                  | 39.7     | 39.8  | 39.6 | 38.2 |
| Soybean meal               | 10.5     | 9.0   | 9.0  | 8.6  |
| Fish meal                  | 7.2      | 7.8   | 7.5  | 7.6  |
| DCP                        | 0.5      | 0.5   | 0.5  | 0.5  |
| Salt                       | 0.3      | 0.3   | 0.3  | 0.3  |
| Stone meal                 | 1.3      | 1.3   | 1.3  | 1.3  |
| Lysine                     | 0.2      | 0.2   | 0.2  | 0.2  |
| Vitamin and mineral premix | 0.3      | 0.3   | 0.3  | 0.3  |
| Total                      | 100      | 100   | 100  | 100  |
| Chemical compositions      |          |       |      |      |
| CP, %                      | 17       | 17    | 17   | 17   |
| ME, kcal/kg                | 3003     | 2989  | 2969 | 2962 |
| Ca%                        | 0.98     | 1.00  | 1.00 | 1.02 |
| P, %                       | 0.73     | 0.70  | 0.73 | 0.72 |
| Lysine, %                  | 1.04     | 1.03  | 1.01 | 1.00 |
| Methionine, %              | 0.46     | 0.46  | 0.45 | 0.45 |

SR: germinated rice; SR0, no replacement of SR; SR1, 15 % replacement; SR2, 30 % replacement; SR3, 45 % replacement; DCP, dicalcium phosphate; Ca, calcium; CP, crude protein; P, phosphate; ME, metabolizable energy. The vitamin and mineral premix were formulated according to local chicken's growth from 6-12 weeks of age.

# Table 2

Chemical composition of sprouted rough rice after 4 days.

| Criteria      | Unit     | Results |
|---------------|----------|---------|
| DM            | %        | 62.1    |
| Crude protein | %        | 10.4    |
| Fat           | %        | 6.95    |
| β-Glucan      | %        | 1.44    |
| Energy        | MJ/kg DM | 12.8    |
| Niacin        | mg/kg    | 7.04    |
| α-Tocopherol  | mg/kg    | <15     |
| Thiamine      | mg/kg    | <1.0    |
| Pyridoxine    | mg/kg    | <1.0    |

All birds were immunised against Newcastle disease, Gumboro disease, and avian influenza at 3, 7, and 14 days of age. The second dose of each vaccination was administered after a 4-week delay. Additionally, the chicken pox vaccine was administered at 9 days of age.

by recording the average live BW of all birds in each pen until they reached the age of 12 weeks. To determine the average feed intake (FI) per bird, the weight of feed refusals was subtracted from the weight of feed delivered (a process performed on a daily basis from week one until the conclusion of week 12 and the resulting difference was divided by the total number of birds housed in each pen. To determine the average feed conversion ratio (FCR), the value of body weight gain over a period of time was compared to the total amount of feed consumed.

# 2.5. Blood profiles and liver enzyme levels

A total of 2 wing vein blood samples from each replication were taken using a sterile syringe and separated into an EDTA tube for chemical analysis before being refrigerated at 4 °C. Blood samples were tested within 48 h of collection. The samples were taken to an animal hospital for biochemical analysis of glucose (GLU), cholesterol (CHO), albumin (ALB), triglyceride (TG), protein (PRO), globulin (GLO), high/low-density lipoprotein cholesterol (H/LDL-c) contents using a Cobas 6000 analyser (Roche Diagnostics, Regensburg, Germany) according to the manufacturer's guideline. Serum samples from blood were also assessed for the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) using the same analyser.

# 2.6. Carcass traits and lymphoid organs

Thirty birds were randomly selected and euthanised at the end of the experiment; one female and one male bird from each replicate. These birds were dissected and eviscerated to assess the size of the carcass characteristics, internal organs, and meat quality. We used a digital scale to measure the carcass weight, breast and thigh weight, internal organ weights (gizzard, intestine, and heart), and immunological organ weights (bursa of Fabricius, thymus, and spleen). The intestines were measured from the end of gizzard to the end of large intestine. The pH of breast flesh was measured after slaughter using a digital pH meter (pH/ ORP/Temperature Laboratory Bench Meter Mi 151, Milwaukee, Brookfield, WI, USA) with a spear-type electrode.

Following slaughter, breast and thigh meat pieces from each bird were cut and weighed. The samples were then immersed in fresh water and cooked in 100 °C for 5 min. Subsequently, the samples were extracted and analysed. Cooking loss was determined by subtracting the final weight (post-cooking) from the original weight (pre-cooking).

# 2.7. Fatty acids in muscles

The fats in the tissue samples were transformed into fatty acid methyl esters using AOAC (Method No. 996.06). In total,  $60 \pm 10$  mg of fat was extracted from the 02 samples from each replication and placed in a 15 mL centrifuge tube. After preparation, the samples were transferred to a

gas chromatography-flame ionisation detector. Saturated, unsaturated, polyunsaturated, and monounsaturated fatty acids were identified and measured using gas chromatography (Agilent Technologies, Santa Clara, CA, USA). The gas chromatograph was outfitted with a split-splitless injector, a flame ionisation detector, and a 30 m fused silica capillary column. The carrier gas employed was helium, with the injector and detector temperatures configured at 250 and 300 °C, correspondingly.

# 2.8. Data analysis

Primary data were processed for initial computations using Microsoft 365 software. Growth performance, and haematological and meat quality data were examined using ANOVA. The means of the treatments were compared using post-hoc multiple comparisons and Tukey's test at a significance level of p < 0.05. Linear and quadratic regression equations were computed for all criteria using SPSS Version 17 for Windows (SPSS Inc., Chicago, IL, USA).

# 3. Results

# 3.1. Growth performance of broilers

Replacing corn meal with germinated rice meal significantly increased the growth performance of broilers (p < 0.05; Table 3). BW at wk 12, BWG from wks 10–12, and BWG from wks 7–12 were highest with in the SR3 group (p < 0.05) compared to the SR0, SR1, and SR2 groups. In contrast, the FI from wks 10–12 and wks 7–12 were highest in the SR0 group compared to the SR1, SR2, and SR3 groups (p < 0.05). In addition, there was a linear increase in BW and BWG from wk 10 to wk 12 and from wk 7 to wk 12 when with the SR increased in the diets (p < 0.05). For FI and FCR from wk 10 to wk 12 and from wk 7 to wk 12, there was a linear increase in SR in the diets (p < 0.05).

# 3.2. Immune organs

Table 6 shows the effect of replacing dietary corn meal with SR on immune organ weight. Thymus and spleen weights were the highest in the SR3 group (p < 0.05). Additionally, there was a linear increase in thymus and spleen weights with increasing SR inclusion (p < 0.05). However, there were no significant differences in the weight of the bursa of Fabricius between treatment groups (p > 0.05).

#### 3.3. Blood chemical parameters and hepatic enzyme activities

SR did not affect the blood profiles or hepatic enzymes of broilers at 12 weeks of age (Table 7). Increased SR in the diet did not significantly affect the blood profiles and hepatic enzyme levels (p > 0.05). Hepatic enzymes showed no significant differences between broilers fed different levels of SR; the lowest level was observed in the SR3 group, but it was not significant (p > 0.05).

# 3.4. Carcass paramenters of broilers

The replacement of corn meal with SR significantly increased the carcass weight and meat quality of broilers (Tables 4 and 5). The SR3 group showed the highest live weight, carcass weight, and breast and thigh weights (p < 0.05) compared with the SR0, SR1, and SR2 groups. SR inclusion increased the weight of the liver and the length of the intestine (p < 0.05), but no significant differences were recorded between SR treatment groups (p > 0.05). Moreover, there was a linear increase in carcass weight, live weight, breast and thigh weight, and the length of the large intestine with increasing SR inclusion (p < 0.05). Regarding carcass quality (Table 5), SR inclusion did not affect cooking loss (p > 0.05). However, the addition of SR decreased the pH of thigh meat; the lowest pH was recorded in the SR3 group (p < 0.05). In addition, there was a linear increase in thigh pH with a decrease in SR inclusion (p < 0.05).

# Table 3

Growth performance of broilers from 6 to 12 weeks of age.

| Criteria <sup>1)</sup>  | Treatments <sup>2)</sup> |                     |                     |                    | SEM   | <i>p</i> -value |        |           |
|-------------------------|--------------------------|---------------------|---------------------|--------------------|-------|-----------------|--------|-----------|
|                         | SR0                      | SR1                 | SR2                 | SR3                |       | Post-Hoc        | Linear | Quadratic |
| BW, g/bird              |                          |                     |                     |                    |       |                 |        |           |
| Week 6                  | 359.7                    | 381.0               | 368.5               | 372.8              | 6.532 | 0.737           | 0.667  | 0.540     |
| Week 12                 | 1149 <sup>c</sup>        | $1210^{bc}$         | 1243 <sup>ab</sup>  | 1314 <sup>a</sup>  | 16.70 | 0.001           | 0.000  | 0.823     |
| BWG, g/bird/day         |                          |                     |                     |                    |       |                 |        |           |
| From 7 to 9 wk          | 17.51                    | 18.05               | 19.61               | 20.09              | 0.395 | 0.051           | 0.007  | 0.965     |
| From 10 to 12 wk        | $20.10^{b}$              | 21.46 <sup>ab</sup> | 22.03 <sup>ab</sup> | 24.77 <sup>a</sup> | 0.616 | 0.042           | 0.007  | 0.529     |
| From 7 to 12 wk         | 18.81 <sup>b</sup>       | 19.75 <sup>b</sup>  | $20.82^{ab}$        | 22.43ª             | 0.387 | 0.002           | 0.000  | 0.569     |
| Feed intake, g/bird/day |                          |                     |                     |                    |       |                 |        |           |
| From 7 to 9 wk          | 62.29                    | 62.84               | 62.06               | 61.91              | 0.149 | 0.128           | 0.141  | 0.227     |
| From 10 to 12 wk        | 75.88 <sup>a</sup>       | 76.47 <sup>b</sup>  | 75.85 <sup>b</sup>  | 74.70 <sup>b</sup> | 0.180 | 0.001           | 0.002  | 0.003     |
| From 7 to 12 wk         | 69.08 <sup>a</sup>       | 69.65 <sup>ab</sup> | 68.95 <sup>b</sup>  | 68.31 <sup>b</sup> | 0.138 | 0.002           | 0.003  | 0.007     |
| FCR                     |                          |                     |                     |                    |       |                 |        |           |
| From 7 to 9 wk          | 3.597                    | 3.489               | 3.169               | 3.123              | 0.074 | 0.052           | 0.007  | 0.812     |
| From 10 to 12 wk        | 3.843 <sup>a</sup>       | 3.617 <sup>ab</sup> | 3.489 <sup>ab</sup> | 3.034 <sup>b</sup> | 0.107 | 0.040           | 0.006  | 0.546     |
| From 7 to 12 wk         | 3.705 <sup>a</sup>       | 3.543 <sup>ab</sup> | 3.323 <sup>b</sup>  | 3.047 <sup>b</sup> | 0.072 | 0.002           | 0.000  | 0.601     |

<sup>a,b,c:</sup> Means with different subscripts are statistically significant (p < 0.05).

<sup>1)</sup> SEM, standard error of the mean; FCR, feed conversion ratio; BWG, body weight gain; BW: body weight; FI, feed intake.

<sup>2)</sup> SR: sprouted rough rice; SR0, no replacement of SR; SR1, 15% replacement; SR2, 30% replacement; SR3, 45% replacement.

# Table 4

Carcass weights of broilers at 12 weeks of age.

| Criteria            | Treatments <sup>1)</sup> |                    |                    |                    | SEM   | <i>p</i> -value |        |           |
|---------------------|--------------------------|--------------------|--------------------|--------------------|-------|-----------------|--------|-----------|
|                     | SR0                      | SR1                | SR2                | SR3                |       | Post-Hoc        | Linear | Quadratic |
| Live weight, g      | 1153 <sup>b</sup>        | 1190 <sup>b</sup>  | 1308 <sup>a</sup>  | 1341 <sup>a</sup>  | 17.96 | 0.000           | 0.000  | 0.901     |
| Carcass weight, g   | 780.6 <sup>b</sup>       | 826.9 <sup>b</sup> | 914.8 <sup>a</sup> | 943.7 <sup>a</sup> | 16.41 | 0.000           | 0.000  | 0.660     |
| Carcass, %          | 67.64                    | 69.43              | 69.89              | 70.39              | 0.601 | 0.416           | 0.121  | 0.598     |
| Breast weight, g    | 139.2 <sup>c</sup>       | 141.5 <sup>c</sup> | 157.8 <sup>b</sup> | 168.7 <sup>a</sup> | 2.623 | 0.000           | 0.000  | 0.010     |
| Breast, %           | 17.88                    | 17.23              | 17.27              | 17.89              | 0.230 | 0.617           | 0.977  | 0.192     |
| Thigh weight, g     | 159.9 <sup>b</sup>       | 164 <sup>b</sup>   | 177.9 <sup>a</sup> | 175.5 <sup>a</sup> | 1.687 | 0.000           | 0.000  | 0.020     |
| Thigh, %            | 20.54                    | 19.93              | 19.48              | 18.63              | 0.293 | 0.123           | 0.020  | 0.825     |
| Heart weight, g     | 5.000                    | 5.250              | 5.350              | 5.800              | 0.168 | 0.416           | 0.111  | 0.769     |
| Gizzard weight, g   | 42.80                    | 38.55              | 41.40              | 42.45              | 0.891 | 0.335           | 0.821  | 0.148     |
| Small intestine, cm | 142.0                    | 146.5              | 152.5              | 140.6              | 1.749 | 0.060           | 0.888  | 0.017     |
| Large intestine, cm | 9.500 <sup>b</sup>       | $10.00^{ab}$       | 10.50 <sup>a</sup> | 10.50 <sup>a</sup> | 0.114 | 0.001           | 0.000  | 0.130     |

<sup>a,b,c:</sup> Means with different subscripts are statistically significant (p < 0.05).

<sup>1)</sup> SR: sprouted rough rice; SR0, no replacement of SR; SR1, 15% replacement; SR2, 30% replacement; SR3, 45% replacement; SEM, standard error of the mean.

# Table 5

Meat quality of broilers at 12 weeks of age.

| Criteria          | Treatments <sup>1)</sup> |                     |                    |                   | SEM   | <i>p</i> -value |        |           |
|-------------------|--------------------------|---------------------|--------------------|-------------------|-------|-----------------|--------|-----------|
|                   | SR0                      | SR1                 | SR2                | SR3               |       | Post-Hoc        | Linear | Quadratic |
| Thigh             |                          |                     |                    |                   |       |                 |        |           |
| Before cooking, g | 5.200                    | 5.650               | 5.350              | 5.400             | 0.064 | 0.086           | 0.575  | 0.105     |
| After cooking, g  | 3.850                    | 4.050               | 3.850              | 4.00              | 0.039 | 0.162           | 0.463  | 0.741     |
| Water loss, %     | 25.51                    | 25.32               | 26.64              | 25.73             | 0.347 | 0.574           | 0.540  | 0.611     |
| Breast            |                          |                     |                    |                   |       |                 |        |           |
| Before cooking, g | 5.350                    | 5.308               | 5.433              | 5.250             | 0.032 | 0.237           | 0.537  | 0.269     |
| After cooking, g  | 4.000                    | 4.150               | 4.200              | 3.900             | 0.048 | 0.094           | 0.533  | 0.190     |
| Water loss, %     | 25.24                    | 22.49               | 25.04              | 25.72             | 0.471 | 0.056           | 0.300  | 0.055     |
| pH                |                          |                     |                    |                   |       |                 |        |           |
| Thigh pH          | 6.033 <sup>a</sup>       | 5.933 <sup>ab</sup> | 6.033 <sup>a</sup> | 5.85 <sup>b</sup> | 0.022 | 0.002           | 0.006  | 0.220     |
| Breast pH         | 6.000                    | 6.033               | 5.950              | 6.050             | 0.024 | 0.481           | 0.758  | 0.493     |

<sup>1)</sup> SR: sprouted rough rice; SR0, no replacement of SR; SR1, 15% replacement; SR2, 30% replacement; SR3, 45% replacement; SEM, standard error of the mean.  $a_{a,b,c}$ : Means with different subscripts are statistically significant (p < 0.05);

# 3.5. Muscle fatty acid profiles

There was a notable disparity in the levels of saturated fatty acid (SFA) and unsaturated fatty acid (USFA), and omega-6 contents, and transfat between treatments (Table 8). The highest performance for overall concentration was observed in the SR2 and SR3 groups (p < 0.05). There was a linear increase in SFA (saturated fatty acid), USFA (unsaturated fatty acid), and MCT (medium-chain triglycerides)

contents, and transfusion with an increase in SR content (p < 0.05). In addition, only C16:0 (palmitic acid), C16:1 (palmitoleic acid), C18:1n9 (*cis*-oleic acid), C18:0 (stearic acid), and C18:3 (alpha-linolenic acid) were detected in the breast tissue, with a threshold of greater than 0.01 %. With increased levels of SR in the diets, C18:0 (stearic acid), C16:0 (palmitic acid), C18:1n9 (*cis*-oleic acid), and C18:3 (alpha-linolenic acid) increased significantly, whereas no effect was recorded for C16:1 (palmitoleic acid).

## Table 6

Immune organ weights of broilers at 12 weeks of age.

| Criteria  | Treatments <sup>1)</sup>                          |   |  |   | SEM                     | <i>p</i> -value         |                         |                         |
|---|---|---|--|---|-------------------------|-------------------------|-------------------------|-------------------------|
|   | SR0   | SR1                                       | SR2  | SR3   |                         | Post-Hoc                | Linear                  | Quadratic               |
| Bursa of Fabricius, g<br>Thymus, g<br>Spleen, g | 1.533<br>5.083 <sup>b</sup><br>2.800 <sup>b</sup> | $1.500 \\ 5.783^{ m ab} \\ 3.800^{ m ab}$ | 1.483<br>6.866 <sup>a</sup><br>4.033 <sup>ab</sup> | 2.000<br>7.050 <sup>a</sup><br>4.950 <sup>a</sup> | 0.088<br>0.236<br>0.297 | 0.112<br>0.000<br>0.004 | 0.074<br>0.000<br>0.012 | 0.110<br>0.000<br>0.939 |

<sup>a,b,c:</sup> Means with different subscripts are statistically significant (p < 0.05).

<sup>1)</sup> SR: sprouted rough rice; SR0, no replacement of SR; SR1, 15% replacement; SR2, 30% replacement; SR3, 45% replacement; SEM, standard error of the mean.

# Table 7

Table 8

Blood profiles and hepatic enzymes of broilers at 12 weeks of age.

|                 | Treatments <sup>2)</sup> |       |       |       | SEM   | <i>p</i> -value |        |           |
|-----------------|--------------------------|-------|-------|-------|-------|-----------------|--------|-----------|
|                 | SR0                      | SR1   | SR2   | SR3   |       | Post-Hoc        | Linear | Quadratic |
| Blood profiles  |                          |       |       |       |       |                 |        |           |
| Protein         | 4286                     | 4516  | 4310  | 4196  | 119.4 | 0.831           | 0.675  | 0.501     |
| Albumin         | 1391                     | 1327  | 1339  | 1542  | 39.33 | 0.192           | 0.179  | 0.091     |
| Globulin        | 2893                     | 3188  | 2971  | 2653  | 134.5 | 0.597           | 0.456  | 0.279     |
| Glucose         | 150.8                    | 152.1 | 152.5 | 134.1 | 5.361 | 0.587           | 0.323  | 0.379     |
| Triglycerides   | 9.600                    | 9.060 | 9.240 | 12.48 | 0.802 | 0.412           | 0.233  | 0.253     |
| Cholesterol     | 69.30                    | 69.36 | 69.72 | 65.94 | 1.127 | 0.633           | 0.360  | 0.417     |
| HDL-c           | 41.64                    | 42.06 | 41.76 | 41.28 | 1.031 | 0.996           | 0.890  | 0.840     |
| LDL-c           | 18.84                    | 18.12 | 18.60 | 19.86 | 0.542 | 0.738           | 0.491  | 0.390     |
| Hepatic enzymes |                          |       |       |       |       |                 |        |           |
| AST             | 210.3                    | 208.1 | 207.8 | 206.1 | 3.810 | 0.986           | 0.817  | 0.781     |
| ALP             | 1519                     | 1200  | 1196  | 1189  | 67.28 | 0.231           | 0.112  | 0.214     |
| ALT             | 6.167                    | 5.833 | 5.333 | 5.333 | 0.155 | 0.156           | 0.143  | 0.091     |

<sup>a,b,c:</sup> Means with different subscripts are statistically significant (p < 0.05);

<sup>1)</sup> SEM, standard error of the mean; alkaline phosphatase, ALP; aspartate aminotransferase, AST; high/low-density lipoprotein, H/LDL; c: cholesterol; alanine aminotransferase, ALT.

<sup>2)</sup> SR: sprouted rough rice; SR0, no replacement of SR; SR1, 15% replacement; SR2, 30% replacement; SR3, 45% replacement.

| Criteria <sup>1)</sup>       | Treatments <sup>2</sup> | )                   |                    |                     | SEM   | <i>p</i> -value |        |           |
|------------------------------|-------------------------|---------------------|--------------------|---------------------|-------|-----------------|--------|-----------|
|                              | SR0                     | SR1                 | SR2                | SR3                 |       | Post-Hoc        | Linear | Quadratio |
| SFA                          | 0.394 <sup>b</sup>      | 0.447 <sup>ab</sup> | 0.598 <sup>a</sup> | 0.603 <sup>a</sup>  | 0.030 | 0.015           | 0.002  | 0.636     |
| PUFA                         | 0.002                   | 0.002               | 0.003              | 0.002               | 0.000 | 0.225           | 0.670  | 0.117     |
| MUFA                         | 0.335                   | 0.345               | 0.399              | 0.407               | 0.015 | 0.271           | 0.065  | 0.972     |
| USFA                         | 0.486 <sup>b</sup>      | 0.567 <sup>ab</sup> | 0.767 <sup>a</sup> | 0.703 <sup>ab</sup> | 0.038 | 0.025           | 0.008  | 0.277     |
| Omega-3                      | 0.003                   | 0.003               | 0.004              | 0.005               | 0.000 | 0.376           | 0.099  | 0.640     |
| Omega-6                      | 0.015                   | 0.016               | 0.016              | 0.018               | 0.000 | 0.303           | 0.249  | 0.274     |
| Omega-9                      | 0.322                   | 0.331               | 0.374              | 0.397               | 0.001 | 0.217           | 0.092  | 0.569     |
| Transfat                     | 0.437 <sup>b</sup>      | 0.501 <sup>ab</sup> | 0.679 <sup>a</sup> | 0.650 <sup>ab</sup> | 0.035 | 0.027           | 0.006  | 0.449     |
| Threshold $> 0.01$           |                         |                     |                    |                     |       |                 |        |           |
| C16:0 (palmitic acid)        | $0.291^{b}$             | 0.335 <sup>ab</sup> | $0.447^{a}$        | 0.439 <sup>a</sup>  | 0.022 | 0.016           | 0.003  | 0.485     |
| C16:1 (palmitoleic acid)     | 0.044                   | 0.060               | 0.080              | 0.045               | 0.006 | 0.103           | 0.628  | 0.031     |
| C18:0 (stearic acid)         | $0.081^{b}$             | 0.089 <sup>ab</sup> | $0.122^{a}$        | $0.127^{a}$         | 0.007 | 0.012           | 0.002  | 0.932     |
| C18:1n9 (cis-oleic acid)     | 0.435 <sup>b</sup>      | 0.499 <sup>ab</sup> | 0.676 <sup>a</sup> | 0.648 <sup>ab</sup> | 0.035 | 0.027           | 0.006  | 0.451     |
| C18:3 (alpha-linolenic acid) | $0.010^{b}$             | 0.011 <sup>ab</sup> | 0.015 <sup>a</sup> | 0.013 <sup>ab</sup> | 0.001 | 0.009           | 0.004  | 0.176     |

<sup>1)</sup> PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; USFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; SEM, standard error of the mean.

<sup>2)</sup> SR: sprouted rough rice; SR0, no replacement of SR; SR1, 15% replacement; SR2, 30% replacement; SR3, 45% replacement.

<sup>a,b,c:</sup> Means with different subscripts are statistically significant (p < 0.05);

# 4. Discussion

The supplementation of diets with SR enhanced the growth indicators of broilers. This is in line with previous reports that the abundance of biochemical compounds in the SR enhances growth performance (Linh et al., 2020; Sultana et al., 2022). The main factor of feed formulation is the digestibility of raw materials. Through the results of this study, it can be assumed that the valuable nutrients and chemical compositions in SR could increase the nutrion absortions, thus, increase growth performance of birds. As similar to the results of our study, Linh et al. (2020) recorded the growth efficacy of broilers has been shown to improve when SR was included at 75 mg/kg of feed. One study observed comparable outcomes, wherein the growth performance of Japanese quail was enhanced by 25, 50, and 100 % substitution of germinated rice for maize (Younis et al., 2019). As stated earlier, the nutritional composition of the seeds sprouted through germination is improved (Johnson and Taverner, 2019; Linh et al., 2020; Oh et al., 2010; Sultana et al., 2022); these seeds are rich in  $\beta$ -glucan, fibre, and  $\gamma$ -oryzanol and  $\gamma$ -amino butyric acid (Oh et al., 2010). SR also contains an abundance of phytonutrients, particularly  $\gamma$ -amino butyric acid, which contributes to its heightened antioxidant properties. Therefore, SR may serve as a viable substitute for feed constituents in poultry production (Likit-trakulwong et al., 2020) and could enhance the growth performance of broilers in this study. Germinated paddy rice is composed of rice husk, a

component known for its substantial ash content. Whole rice hulls can be incorporated into poultry feeds as a source of insoluble fibre, potentially improving growth and uniformity (Incharoen et al., 2021). Evidence has shown that the production of starch-breaking enzymes, such as  $\alpha$ -amylase and  $\alpha$ -glucosidase, can be triggered in scutellum and aleurone cells during the process of sprouting (Sultana et al., 2022). This enzyme production could potentially promote the growth of birds. Additionally, β-glucan content in SR has been found to enhance microbial activity and gastrointestinal tract capacity, potentially leading to an increased growth rate (Qui and Linh, 2023). While the exact mechanisms underlying the enhanced growth performance of broiler chickens following grain sprouting supplementation in the diet remain elusive, it is plausible that the sprouting process enhances the functional and nutritional qualities of bioactive compounds present in sprouted grains, facilitates the spontaneous synthesis of diverse nutrients, degrades certain antinutritional components in the feed, and generates advantageous enzymes to enhance digestion (Sultana et al., 2022).

The lymphoid organs, which contribute to immune function and growth performance, may demonstrate enlargement in order to enhance the health and growth of the broiler. In this study, SR supplementation enhanced the carcass characteristics of broiler chickens. This could be due to proteins or other substances in the diets. According to previous findings, β-glucan (found in SR) stimulates microbial activity in the gastrointestinal tract, which could accelerate the synthesis of muscle proteins and, consequently, increases the weight of the breast and thigh muscles (Azrinnahar et al., 2021).  $\beta$ -glucan in the gut can also inhibit the adhesion of pathogenic microorganisms. This may have resulted in reduced nutrient competition between pathogens and the host, thereby enhancing the ability of broilers to efficiently utilise nutrients for muscle growth (Qui and Linh, 2023); comparable results have been previously reported (Sugiharto et al., 2021). An additional study found that the inclusion of β-glucan in the diet had no impact on carcass properties, dressing percentage, stomach weight, or gizzard weight (Amer et al., 2023). It has also been demonstrated that sprouted cereals and cornbased diets do not affect the proportion of digestible feed in broilers (Younis et al., 2019). Similarly, the accessibility of  $\beta$ -glucan in the SR did not deviate from these standards. No alterations in cooking loss of breast and thigh meat were observed in this study. Identical findings have been reported previously, indicating that neither  $\beta$ -glucan from natural source nor commercial β-glucan affected cooking loss (Qui and Linh, 2023). On the other hand, it has been demonstrated that diet fortification with  $\beta$ -glucan can decrease water loss during cooking (Zhang et al., 2020). Unexpectedly, the pH of the thigh meat varied among treatments and this variance was greater than that of the water loss observed in the breast muscle. Variations in outcomes may arise because of variations in study conditions, bird strain used, and supplement formulations. An alternative explanation for the greater water absorption capacity of myofibrillar proteins in breast muscles relative to thigh muscles is that the two types of muscles have distinct chemical properties, including varying degrees of dietary protein denaturation (Choe and Kim, 2020).

Supplemental SR could lead to the increased contents of β-glucan (Amer et al., 2023; Qui and Linh, 2023) in the diet, and thus, might stimulate the immune system of broilers through enlargement of the immune organs, including the bursa of Fabricius, thymus, and spleen. In this study, enlargement of the thymus and spleen could be related to nutrient digestibility and the immunity of broilers via mechanisms affected by substances in SR. The presence of  $\beta$ -glucan in SR may have enhanced the generation of precursor cells in the bone marrow, leading to an increased migration of immunocytes into the lymphoid organs. The immunological response to feed supplementation or replacement in broiler chickens differs depending on the lymphoid compartment, as each organ possesses distinct structure and function. Modifications in the size of the thymus may be linked to alterations in the functionality of the lymphoid organ (Jacob and Pescatore, 2017). Additionally, the increase in the number of lymphatic organs could be partly related to external factors that were not measured, such as inflammation and

disease. Further investigations are required to focus on the immune response to SR.

Muscle fatty acids were similarly altered after the substitution of SR in the diets. Fatty acid composition influences the flavour of meat. Increased concentrations of saturated and monounsaturated fatty acids lead to favourable evaluations in terms of tenderness, juiciness, flavour, and overall satisfaction. A high content of polyunsaturated fatty acids, on the other hand, results in mushy and acidic carcass fat, which imparts an unfavourable odour and texture to the meat. The chemical composition of chicken meat influences its processing and storage (Amer et al., 2023). To the best of our knowledge, the impact of SR on muscle fatty acids in broilers has not yet been investigated. The biochemical constituents of the SR may have accounted for the changes we observed in the fatty acid profiles in the muscle tissue. It has been demonstrated that quails supplemented with SR diets contained less cholesterol (Younis et al., 2019); this may cause alterations in the composition of fatty acid of meat. It has been suggested that  $\beta$ -glucans in the diet may influence lipid metabolism (Amer et al., 2023). The possibility of decreased oxidative stress in broilers fed germinated pulses has been supported by work reporting a decrease in lipid peroxidation and enhanced activities of antioxidant enzymes in growing poultry supplemented with β-glucan (Amer et al., 2023; Rama Rao et al., 2018); this could substantially affect the flesh quality. Furthermore, the inclusion of  $\beta$ -glucan in poultry diets (Qui and Linh, 2023), and other substances examined in SR during this investigation, may provide an additional rationale for the observed decrease in oxidative stress and its impact on lipid metabolism.

Scientists worldwide have become increasingly concerned regarding the effects of sprouted grains on plasma lipid profiles. In this study, SR had a beneficial effect on muscle fatty acids, but not on serum lipid profiles. Animal nutrition status, pathology, and physiology are reflected in blood lipid parameters. Fat and cholesterol in data that are not different due to the function of maintenance or regulation of the the body. Besides, changes in haematological parameters can be used to ascertain the impact of additives and dietary nutritional components on living organisms (Qui and Linh, 2023). The mechanisms by which dietary SR act have not been previously described. In this study, the biochemical profiles of blood and hepatic enzyme activities in broiler livers were not affected by SR inclusion. This is consistent with a previous report showing that blood serum triglyceride and cholesterol levels of birds fed a commercial diet remained unaltered for the duration of the experiment (Sultana et al., 2022). The reason for this lack of change is unknown; however, the serum levels were higher without statistical significance, this group had the highest overall body weight, feed intake, and feed utilisation, which may indicate that they consumed and/or utilised a greater quantity of cholesterol and triglycerides, ultimately contributing to their elevated serum levels. Owing to the lack of information regarding the precise chemical composition of the commercial feed used, the presence of feed additives may have contributed to the elevated cholesterol and triglyceride levels observed. On the other hand, another study demonstrated that the incorporation of germinated sorghum into the diet of Japanese quail resulted in a substantial decrease in serum triglyceride and cholesterol levels (Younis et al., 2019). In Japanese quails fed diets added with varying concentrations of germinated Moringa oleifera seed, HDL levels were found to increase, while total blood serum cholesterol and LDL levels decreased (Moustafa, 2016). There was an absence of data on the activity of hepatic enzymes affected by SR in diets. This study suggests that the substitution of SR had no impact on the health of poultry, particularly with regard to liver function. This study is the first to report data regarding the effect of SR on hepatic enzymes in broilers. According to previous studies, an increase in serum AST and ALT activity can be indicative of cellular damage to hepatocytes and cardiac muscles. Conversely, hypoproteinaemia could be a consequence of hepatocyte injury, wherein the synthesis of plasma proteins fails; the liver is the site of albumin synthesis. Renal diseases that cause protein loss and congestive heart failure can contribute to hypoproteinaemia. Hepatitis and cirrhosis are

simultaneously associated with hyperglobulinemia (Sharma et al., 2015). All other blood parameters, including serum AST and ALT activities, were unaffected by dietary replacement with SR. One potential explanation for these findings is that SR did not appear to have any detrimental effects on hepatic enzymes or blood lipid synthesis. Additionally, the quantities of  $\beta$ -glucan or other bioactive components present in the SR of this study were insufficient to significantly alter blood parameters or reduce cholesterol levels in broilers of various breeds.

# 5. Conclusion

It can be inferred that the growth and health of broilers were enhanced by increased dietary levels of SR in place of maize; the highest growth performance was observed at a replacement rate of 45 %. This study documented noteworthy alterations in the carcass weights of broilers, including the whole body, thighs, and breasts with 45 % SR. However, no significant disparities were observed in the carcass percentage, culinary loss of certain organs, or organs when the SR content of the diets increased. Additionally, the SR replacement with 45 % linearly resulted in enlargement of the immune organs (thymus and spleen). This study also found linear increases in the fatty acid content of the breast muscle in response to an increased dietary SR from 0 to 45 %. However, there were no significant differences in the blood profiles between treatment groups.

# Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

# Acknowledgments

This study was fully funded by Tra Vinh University under grant contract number 137/2022/HĐ.HĐKH&ĐT-ĐHTV.

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