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IMPACT OF *TRICHODERMA ASPERELLUM* FERMENTED AGRICULTURAL WASTE-BASED FEED ON BROILER CHICKEN GROWTH AND MEAT QUALITY

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ABSTRACT

The demand for poultry products is increasing due to the rising global population. This has led to high prices and inadequacy of traditional poultry feed ingredients, creating a need to explore alternative feed sources like agricultural wastes. The present study was conducted to assess the effect of *Trichoderma asperellum*-fermented agricultural waste-based feed on the growth and meat quality of broiler. Wheat bran and rice husk were selected as agricultural waste-based substrates for solid state fermentation (SSF). A total of 25 one-day old broiler chicks were distributed into 5 treatment groups randomly: group 1 was fed completely on basic feed mixture (control), group 2 was fed on diet with 10% unfermented wheat bran (WB), group 3 was fed on diet with 10% fermented wheat bran (FWB), group 4 was fed on diet with 10% unfermented rice husk (RH) and group 5 was fed on diet with 10% fermented rice husk (FRH). The experiment was conducted for 5 weeks. Nutrient analysis showed that solid-state fermentation (SSF) improved the protein and carbohydrate content of the substrates. Statistical analysis of growth performance showed that there was a significant ($P < 0.05$) improvement in body weight in the group fed on diet with 10% fermented wheat bran (FWB) compared to control group. The group fed on 10% fermented rice husk (FRH) maintained a similar body weight as compared to control group. The groups fed on 10% unfermented wheat bran (WB) and 10% unfermented rice husk (RH) had lower body weight than control group. No significant changes were observed in meat quality parameters among all the treatment groups. In conclusion, fermenting the wheat barn through fungus *Trichoderma asperellum* not only improved the nutrient content by degrading complex components but also had a positive effect on chicken growth. This study suggests that wheat bran fermented by *Trichoderma asperellum* can be added to broiler diets at up to 10% without any deleterious effect on growth and meat quality.

1. INTRODUCTION

The poultry industry is experiencing feed price fluctuations due to rising costs of key feed ingredients prompting poultry nutritionists to explore alternative feed sources (Sugiharto et al., 2017). Some developing nations are producing various alternative ingredients such as wheat bran, rice bran, cottonseed meal, palm kernel cake and copra meal derived from agricultural wastes and byproducts. These feedstuffs are more affordable compared to main grain crops. However, their high fibre contents limit their usefulness for monogastric animals (Oguri et al., 2013; Chu et al., 2017).

For example, wheat bran and rice husk are among such ingredients with restricted applications in monogastric diets. The outer coat of seed from flour mills is known as wheat bran (Saini et al., 2023). It has the potential to replace yellow corn in poultry diets partially or completely (Alshelmani et al., 2021). WB includes a significant quantity of protein (160 g/kg), complete dietary fibre (451 g/kg), carbohydrates (177 g/kg), minerals (61.5 g/kg) and fat (47g/kg) (Teng et al., 2017; Junejo et al., 2019). It has high fibre content but low metabolizable energy (ME), thus its use in poultry diets is limited. Its nutrient content can be improved by microbial fermentation. It has been found that replacement of 10% basal diet with fermented wheat

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bran can be incorporated to broiler diets without any deleterious effect (Chu et al., 2017).

Rice husk is an agricultural waste generated during rice grain milling. Currently, large piles of rice husks continue to accumulate in many milling areas, where they are often burned for heat or discarded as waste. This disposal method poses significant environmental risks, including pollution and resource wastage. The recent trend in livestock farming is to utilize agricultural waste as substitute feed ingredients in broiler and other animal feeding trials. Although rice husk has long been recognized as a potential feed ingredient, its high silica content, high fiber levels, and harsh texture make it difficult to degrade, limiting its application in animal feed (Ikpe and Oko, 2019). Fermentation is a technique that enhances nutritional value of feed ingredients by reducing crude fiber and antinutritional contents (Predescu et al., 2024). To improve the nutrient content of agricultural wastes and by-products, researchers have been using fungal inoculums in recent years (Sharma and Arora, 2011; Choudhary et al., 2016). Recent studies have employed various Trichoderma species to ferment wheat bran and rice husk, breaking down complex compounds such as cellulose, hemicellulose, lignin, and non-starch polysaccharides into simpler forms. This process enhances their digestibility for broilers and improves their nutrient content (Aderolu et al., 2007; Chu et al., 2017).

Trichoderma are free living and filamentous fungi. They are usually found on decomposing plants, wood, and in the plant rhizosphere, where they provoke general tolerance against pathogens (Afzal et al., 2021; Jangir et al., 2017). Species of Trichoderma are good manufactures of cellulases. They have strong penetration ability into non-soluble substrates and are therefore considered highly suitable for fermenting materials containing lignin and cellulose (Elmasry et al., 2017). Chiang et al. (2009) described that solid state fermentation enhanced the digestibility of nutrients, increased lactobacilli count in the large intestine, improved the gut morphology of broiler chickens and reduced the cost of poultry farming. Alshelmani et al. (2017) stated that broilers fed on feed compositions accompanied by *Paenibacillus polymyxa*-fermented palm kernel cake exhibited improved growth rates and meat quality. Likewise, Chu et al. (2017) pointed out that *Trichoderma pseudokoningii*-fermented wheat bran supplemented diets improved the growth rate and intestinal morphology of broilers. Similarly, Gungor and Erener (2020) showed that broilers given a diet supplemented with 1% *Aspergillus niger* -fermented sour cherry kernel improved weight gain and feed conversion ratio in broilers. (Aderolu et al., 2007) indicated that *Trichoderma viride* fermented rice husk showed improved degradation of cellulosic content and increased energy content. Elmasry et al. (2017) indicated that wheat bran fermented with *Trichoderma longibrachiatum* as 10% feed additive improved the growth performance and feed consumption of broilers than control group. According to Hatta et al. (2014) broilers fed diets containing copra meal fermented with *Trichoderma viride* exhibited similar growth performance to the control group. Similarly, Marcinčák et al. (2018) described that corn meal fermented with *Umbelopsis isabellina* improved the growth and quality of meat of broilers fed on it as compared to control group.
Only a few microbes have been investigated so far to improve the nutritional value and digestibility of agricultural waste-based feed ingredients. In the current study, the potential of a lignocellulose degrading fungus *Trichoderma asperellum* was evaluated to improve the nutritive value of agro-waste products and its effects on broiler growth and meat quality were assessed.

2. MATERIALS AND METHODS

Collection and culturing of fungus

The previously isolated and identified fungus, *Trichoderma asperellum*, was procured from the Department of Biology, Lahore Garrison University. Potato dextrose agar (PDA) was utilized as the growth medium. The media was prepared and poured into petri plates under sterile conditions and allowed to solidify. The fungal inoculum was inoculated onto PDA plates with the help of sterile inoculating loop and incubated at 25-30°C for 7 days.

Collection of substrate samples

Wheat bran and rice husk were selected as substrates for solid state fermentation. Both were purchased from the local market and stored under ambient conditions until fermentation.

Pretreatment, Solid state fermentation (SSF), and Nutrient analysis

Aluminum boxes each containing 100 grams of wheat bran and rice husk were filled with deionized water to achieve 50% moisture content, mixed thoroughly, packed, and autoclaved. The boxes were then cooled to room temperature (Chu et al., 2017). For solid-state fermentation, a sterile saline solution was added to cultured PDA plates, and fungal spores of *Trichoderma asperellum* were harvested and suspended at a concentration of 1×10^5 to 1×10^7 spores per ml. Each kilogram of wheat bran and rice husk was inoculated with 200 ml of spore suspension, packed, and incubated at 30°C for two weeks.

Post-incubation, the samples were autoclaved, washed, sun-dried, and stored at room temperature (Elmasry et al., 2017). For nutrient analysis, the fermented and unfermented samples were mixed with phosphate buffer, ground, and centrifuged. The supernatants were collected for protein and carbohydrate quantification. Protein content was measured using the Lowry method, with absorbance recorded at 660 nm (Satpathy et al., 2020), while carbohydrates content was determined using the Anthrone test, with absorbance recorded at 630 nm. Results were expressed as milligrams of protein or carbohydrate per gram of substrate.

Experimental birds, Housing, and Categorization

Twenty-five healthy one-day broiler chicks were separated from the flock and housed in a local animal shed in Gujranwala city. The chicks were weighed and randomly assigned to five dietary groups: Group 1 was fed a basic feed mixture (control), Group 2 received a diet with 10% unfermented wheat bran (WB), Group 3 received a diet with 10% fermented wheat bran (FWB), Group 4 was fed a diet with 10% unfermented rice husk (RH) and Group 5 received a diet with 10% fermented rice husk (FRH). The experiment was conducted over 35 days, during which the chicks had ad libitum access to feed and water.

Statistical analysis

The data for total protein and carbohydrate content was subjected to two sample T-test, assuming equal variances, in SPSS software to evaluate the statistical effect of microbial fermentation on wheat bran and rice husk. Data on body weight and meat quality were subjected to one-way ANOVA using SPSS software to assess the effects of experimental diets on the treatment groups. Significant differences between treatment groups were considered at a significance level of $P < 0.05$.

3. RESULTS AND DISCUSSION

Protein analysis

The protein content of unfermented and *Trichoderma asperellum*-fermented wheat bran and rice husk was analyzed through Lowry method, with results presented in Table 1. Fermentation with *T. asperellum* significantly increased the protein content of wheat bran from 19.06±0.13 mg/g to 54.51±0.09 mg/g, and rice husk from 10.56±0.06 mg/g to 10.80±0.06 mg/g. According to the t-test analysis, the increase in protein concentration for both fermented wheat bran and fermented rice husk compared to their unfermented counterparts was statistically significant ($P < 0.05$), as shown in Table 1.

Carbohydrate analysis

The carbohydrate content of unfermented and *Trichoderma asperellum*-fermented wheat bran and rice husk was analyzed through anthrone test, with results presented in Table 2. Solid-state fermentation with *Trichoderma asperellum* increased the carbohydrate content of wheat bran from 16.45±0.07 mg/g to 22.58±0.14 mg/g and rice husk increased from 6.45±0.06 mg/g to 6.50±0.12 mg/g. According to t-test analysis, the increase in carbohydrate concentration for fermented wheat bran compared to unfermented wheat bran was statistically significant ($P < 0.05$). However, for rice husk, the increase in carbohydrate concentration was not statistically significant ($P > 0.05$), as shown in Table 2.

Effect of designed feed mixtures on the growth performance

The effects of the designed feed mixtures on the body weight of broiler chickens are presented in Table 3. One-way ANOVA revealed statistically significant differences in the body weights of chicks among the treatment groups at weeks 2, 3, 4, and 5 ($P < 0.05$). A least significant difference (LSD) post hoc test showed that the group fed a diet containing standard feed with 10% fermented wheat bran (FWB) exhibited significantly higher body weight compared to the control and other treatment groups after five weeks. This result highlights the positive effect of fermented wheat bran. Figure 1 provides a graphical representation of the effects of the designed feed mixtures on the body weight of broiler chicks from week 1 to week 5.

Effect of designed feed mixtures on the meat quality

The parameters used to measure meat quality were pH and colour with results presented in Table 4. The L*, a*, and b* values were used as indicators of breast meat colour. Chicken meat colour primarily depends on lightness values. One-way ANOVA revealed no significant differences in breast meat colour and pH among the treatment groups, indicating that the feed supplements did not result in any significant changes in meat quality. Figures 2 and 3 provide graphical representations of the pH and color differences between the treatment groups. The current study assessed the effect of fungal fermented agricultural waste-based feed on broiler chicken growth and meat quality, using the fungus *Trichoderma asperellum* (previously isolated and identified). Fermentation of wheat bran with *T. asperellum* increased protein content from 19.06 mg/g to 54.51 mg/g, and rice husk protein content increased from 10.56 mg/g to 10.80 mg/g. Fungi grow on substrates and secrete various cellulose-degrading enzymes that convert non-starch polysaccharides into simple sugars. These sugars are absorbed by the fungus to stimulate metabolic activities and biosynthesis, leading to an increase in protein content (Chu et al., 2017). Furthermore, the fungi's ability to produce enzymes capable of hydrolyzing starch and non-starch polysaccharides into monosaccharides contributes to increased protein proportion, as monosaccharides are easily converted into protein (Bayitse et al., 2015). Carbohydrate content (simple sugars) of wheat bran increased from 16.45 mg/g to 22.58 mg/g after solid-state fermentation (SSF) with *T. asperellum*, while rice husk only showed a minor increase from 6.45 mg/g to 6.50 mg/g. The enzymatic activity during SSF efficiently breaks down lignocellulosic material (crude fiber) into reducing sugars, which are more digestible for poultry than crude fiber (Lin *et al*., 2018).

These findings align with previous studies, such as Chu et al. (2017), who reported that *T. pseudokoningii*-fermented wheat bran increased by reducing sugars and crude protein content. Similarly, Omwango et al. (2013) observed that pineapple waste fermented with *T. viride* enhanced crude protein content while reducing crude fiber. Ezekiel and Aworh (2013) showed that *T. viride*-fermented cassava peel significantly improved crude protein content while reducing crude fiber. Aderolu et al. (2007) demonstrated that *T. viride*-fermented rice husk reduced NDF and ADF (crude fiber) content. SSF was employed to ferment wheat bran and rice husk, chosen based on substrate characteristics. SSF provides better yield and simplifies downstream processing compared to submerged fermentation (Sugiharto and Ranjitkar, 2019). Cellulose, starch, and lignin are commonly used in SSF as carbon sources, with bacteria and yeast typically growing on the substrate surface, while fungi invade the substrate through mycelium (Srivastava et al., 2019).

In this study, the treatment group fed a diet with 10% fermented wheat bran (FWB) showed a significant increase in body weight (BW) after 5 weeks, reaching 1380 g/bird, compared to the control group (1304 g/bird) and the group fed 10% unfermented wheat bran (WB) (1296.2 g/bird). These results align with Elmasry et al. (2017), who reported a significant increase in BW in broilers fed on wheat bran fermented with *T. longibrachiatum* (SF1). Gungor and Erener (2020) also found improved growth in broilers fed sour cherry kernel fermented with *Aspergillus niger*. However, Hatta et al. (2014) reported that the growth rate of broilers fed fermented wheat bran was like the control group. Similarly, Chuang et al. (2019) found no significant change in the growth of broilers fed a diet with Aspergillus oryzae and Saccharomyces cerevisiae-fermented wheat bran.

In contrast, the treatment groups fed 10% rice husk (RH) and 10% fermented rice husk (FRH) did not show significant changes in body weight compared to the control group. The group fed 10% rice husk (RH) had a slightly lower body weight (1293.4 g/bird) than the control group (1304 g/bird), but this difference was not statistically significant. The group fed 10% fermented rice husk (FRH) had a body weight (1311 g/bird) like the control group (1304 g/bird). These results are consistent with Ikpe and Oko (2019), who reported no significant change in body weight in groups fed rice husk fermented with molasses syrup.
Post-slaughter, muscle pH is an important factor influencing meat quality traits, such as color, water holding capacity, and tenderness. The normal pH for breast muscle is above 5.70 (Alshelmani et al., 2016). In the present study, no significant differences were observed in pH between treatment groups (10% WB, 10% FWB, 10% RH, and 10% FRH) and the control group. However, the pH of the 10% rice husk group was slightly lower than the control group. The pH range was from 5.58 to 5.82 across all treatment groups. These findings are in line with Alshelmani et al. (2016), who reported no significant difference in breast muscle pH when palm kernel cake was treated with *Paenibacillus polymyxa* (ATCC 842). Meat color can be influenced by various factors, including nutrition, sex, age, strain, processing methods, intramuscular fat, and pH. A notable relationship exists between pH and meat color, where a decrease in pH can lead to protein denaturation, resulting in pale color. Lightness (L*) values are commonly used to assess meat color. In the present study, breast meat color across all treatment groups fell within the normal range ($56 \leq L^* \leq 62$), indicating no significant differences in color among the groups. These results are consistent with Ashayerizadeh et al. (2018), who reported no significant effect on breast meat color and pH in broilers fed rapeseed meal fermented with *Aspergillus niger*, *Lactobacillus acidophilus*, and *Bacillus subtilis*.

4. CONCLUSION

It was concluded that *Trichoderma asperellum*-fermented wheat bran and rice husk can serve as effective feed ingredients in poultry diets. *Trichoderma asperellum*-fermented wheat bran can be included in broiler diets up to 10% without negatively affecting growth or meat quality.

5. CONFLICT OF INTEREST

All authors declare that they have no conflict of interest regarding the publication of this article.

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Table 1. Protein content of fermented and unfermented substrates

Substrate samples	Protein concentration (mg/g)	P-value
Unfermented wheat bran	19.06±0.13 mg	0.000
Trichoderma asperellum fermented wheat bran	54.51±0.09 mg	
Unfermented rice husk	10.56±0.06 mg	0.049
Trichoderma asperellum fermented rice husk	10.80±0.06 mg	

Values symbolize the average (mean) of 3 replicates of each treatment group along with standard error.

Table 2. Carbohydrate content of fermented and unfermented substrates

Substrate samples	Carbohydrate concentration (mg/g)	P-value
Unfermented wheat bran	16.45±0.07 mg	0.000
Trichoderma asperellum fermented wheat bran	22.58±0.14 mg	
Unfermented rice husk	6.45±0.06 mg	0.718
Trichoderma asperellum fermented rice husk	6.50±0.12 mg	

Values symbolize the average (mean) of 3 replicates of each treatment group along with standard error.

Table 3. Effect of designed feed mixtures on average weekly live body weight of broiler chickens

	DESIGNED FEED MIXTURES					
	T1	**T2**	**T3**	**T4**	**T5**	
Weeks	**Control**	**Diet with 10% WB**	**Diet with 10% FWB**	**Diet with 10% RH**	**Diet with 10% FRH**	**P-value**
week 0	43.14±0.51	43.60±0.42	43.54±0.48	43.34±0.54	43.56±0.59	0.965
week 1	138.8±3.60	134±1.70	135.6±1.50	131.6±2.48	130.6±3.76	0.317
week 2	337.4^{ab}±5.89	323.2^{b}±14.0	370.8^{a}±14.7	306.2^{b}±16.7	310.6^{b}±7.15	0.011
week 3	618^{b}±8.60	616.4^{b}±11.7	672^{a}±8.60	606.2^{b}±19.0	614.2^{b}±13.3	0.012
week 4	900.4^{b}±21.7	943.4^{ab}±22.4	989.2^{a}±11.7	931.4^{b}±11.8	928.4^{b}±11.4	0.019
week 5	1304^{b}±23.3	1296.2^{b}±17.3	1380^{a}±11.4	1293.4^{b}±16.5	1311.8^{b}±15.1	0.011

Values symbolize the average (mean) of 5 models of each treatment group along with standard error. Means within same rows with different superscript letters (ab) are significantly different (p<0.05). WB: wheat bran, FWB: fermented wheat bran, RH: rice husk, FRH: fermented rice husk.

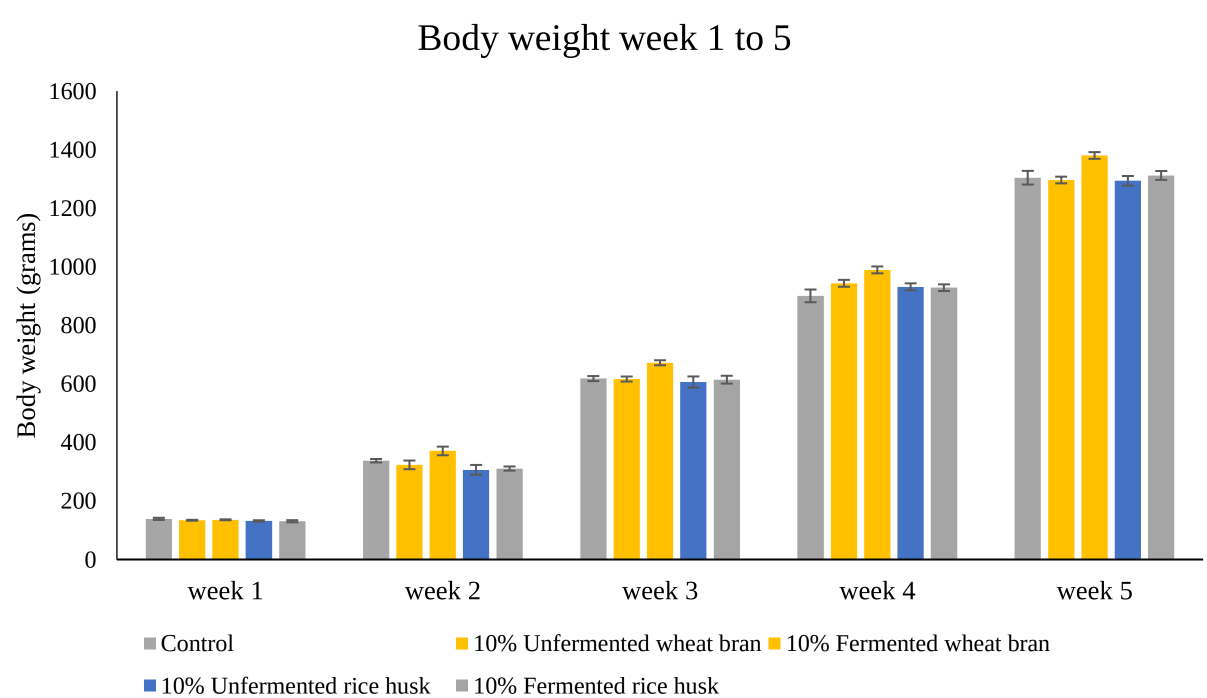

Figure 1. Effect of designed feed mixtures on the body weight of broiler chicks during the experiment study Each bar represents average body weight along with standard error (n=5). Fermented wheat bran-based feed improved the body weight in each successive week shown with yellow bar.

Table 4: Effect of designed feed mixtures on breast meat colour and pH of broiler chicken

	DESIGNED FEED MIXTURES					
Parameters	**Control**	**WB**	**FWB**	**RH**	**FRH**	**SEM**	**P-value**
Breast pH	5.82	5.71	5.77	5.58	5.71	0.021	0.261
L* (Lightness)	56.97	57.22	57.36	56.29	58.39	0.239	0.083
a* (redness)	3.74	3.78	4.09	3.94	3.76	0.120	0.890
b* (yellowness)	6.49	6.42	6.84	7.26	6.54	0.112	0.092

Values symbolize the average (mean) of 5 models of each treatment group. WB: wheat bran, FWB: fermented wheat bran, RH: rice husk, FRH: fermented rice husk. SEM: standard error of mean.

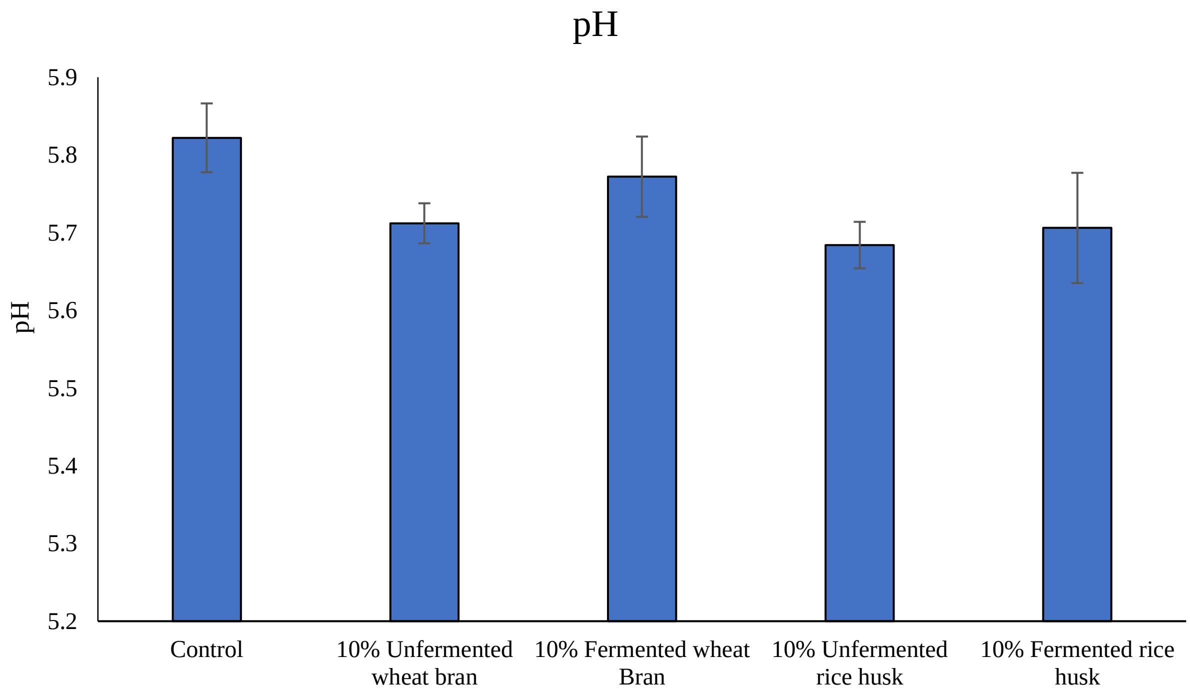

Figure 2. Effect of experimental diets on the pH of broiler chicken. Each bar represents average pH along with standard error (n=5). No significant difference was detected between treatment groups (Least significant difference, P<0.05).

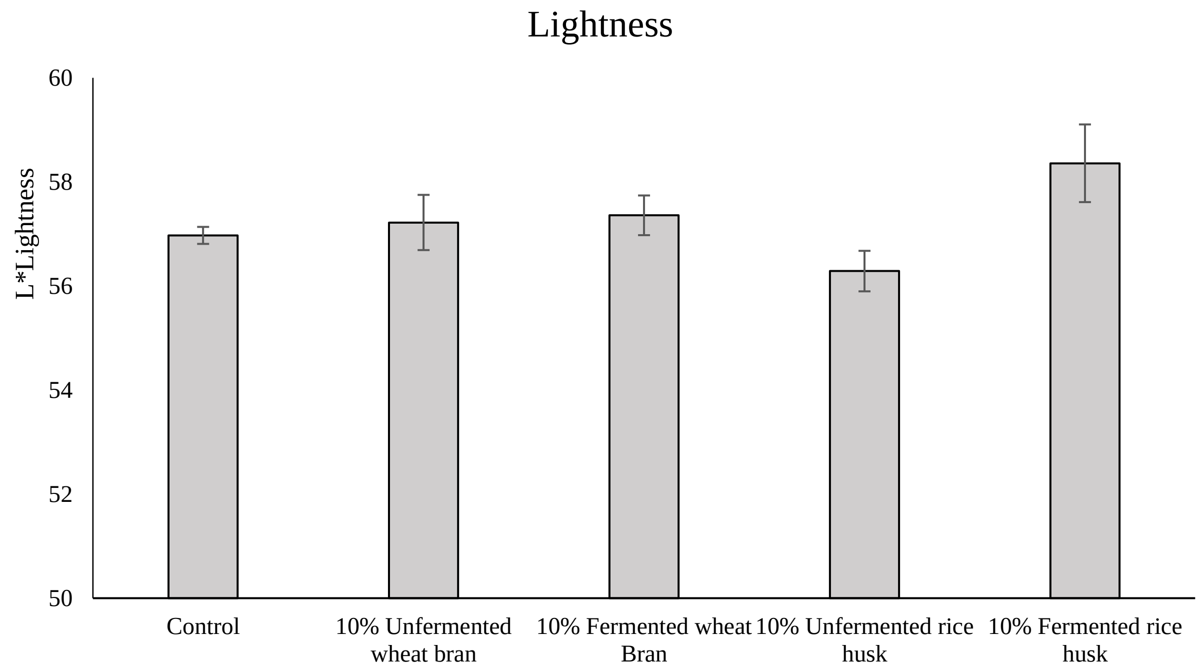

Figure 3. Effect of experimental diets on the breast meat colour (L*) of broiler chicken. Each bar represents average values of lightness along with standard error (n=5). No significant difference was detected between treatment groups (Least significant difference, P<0.05).