https://doi.org/10.4038/vingnanam.v17i2.4195

Different heat treatments in rice processing affect the nutritional and microbiological aspects of rice bran incorporated poultry feed

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Abstract – Rice bran, a byproduct of the rice milling industry, is used for poultry feeding in Sri Lanka. High fiber content, rancidity development, enzyme inhibitors, and high mold growth, limit its potential to use as a poultry feed ingredient. The present study was carried out to evaluate the effect of different heat treatments; parboiling, autoclaving, microwave heating, and dry heating on the nutritional value and microbiological safety of rice bran incorporated poultry feed. Two rice varieties, BG 358 and Gonabaru were used. Control and four treatments as crude (T1), parboiled (T2), autoclaved (T3), dry heated (T4), and microwave heated (T5) rice bran of each rice variety was incorporated into the formulated poultry feed. Changes in nutritional value with different treatments were compared by analyzing crude protein, total lipid, and crude fiber contents. Moisture content, and total fungal and bacterial counts were measured during the four- week storage. Data were analyzed in a completely randomized design using MINITAB software. Crude protein, crude fiber, and moisture content were significantly reduced ($p<0.05$) in heat-treated rice bran incorporated poultry feed of two tested rice varieties, compared to non-heated control. Significantly high lipid content ($p<0.05$) and lowest fungal and bacterial counts were shown in T5 and independent of the tested rice variety. Although the values are different, the effect of the heat treatments on the nutritional quality and microbiological safety of rice bran incorporated poultry feed is affected in the same manner for two tested rice varieties.

Keywords: Heat treatment, Nutritional value, Poultry feed, Rice bran

1. Introduction

Rice bran is the hard outer layer of the rice grain that is removed in the processing of brown rice into white rice, and it accounts for 8 to 10% of the whole grain (Wani *et al*., 2012). The major cereal by-product available for animal feeding in rice-growing countries is rice bran (Gul *et al*., 2015). The nutrients available in the rice bran contain protein (13.20 -17.13%), fat (14.00 to 22.90%), carbohydrate (16.10%), fiber (9.50 - 13.20%), vitamins (vitamin E and vitamin B), antioxidants and minerals (magnesium, potassium, phosphorus, iron, manganese, and zinc) (Sharif *et al*., 2014). Rice bran protein consists of important amino acids such as higher contents of lysine (4.31%), threonine and isoleucine (Sharif *et al*., 2014).

Poultry feed is feed for farm poultry, including chicken, ducks, geese, and other domestic birds (Mujahid *et al*., 2013). Generally, poultry requires a sufficient amount of protein and carbohydrates along with necessary vitamins, dietary minerals, and an adequate supply of water for healthy growth. In Sri Lanka, rice bran is commonly used for poultry feeding due to its high availability and low cost. Also, poultry feed must be remained clean and dry as contaminated feed can infect poultry. Mycotoxin poisoning is one of the most common toxicities in poultry (Sanchez *et al*., 2019). These diseases can be avoided with proper maintenance of the feed and feeder, as it is necessary to maintain a safe level of microbes in poultry feed.

The incorporation of rice bran into poultry feed is critical as the raw rice bran has a very short shelf life due to its high-fat content and potent lipase enzyme (Saikia and Deka, 2011). This lipase enzyme rapidly hydrolyzes oil to free fatty acids (FFA) and glycerol. Once, the bran is separated from the rice kernel, it increases acidity and causes odor and rancid flavor. Lipoxygenase and peroxidase are the enzymes that can be found in the rice bran. The enzymes play a key role in the oxidative rancidity of the oil present in rice bran. This may result in problems in palatability and growth depression in poultry (Osunbami *et al*., 2021).

The antinutritional compounds present in rice bran, such as trypsin inhibitors, pepsin inhibitors, hemagglutinins, phytates, and an anti-thiamine, further aggravates the poor performance of poultry (Lamid *et al*., 2014). High moisture content and mould growth in raw rice bran reduce its keeping quality. These problems are associated with rice bran and limit its potential to use as poultry feed.

However, it is necessary to stabilize the rice bran just after milling and before storage to use as a valuable feed ingredient. Primary means for rice bran stabilization include deactivating the enzymes through heat treatments, such as hot air oven or microwave heating, ohmic heating and steaming (Sharma *et al*., 2004). In Sri Lanka, a few studies have been conducted to determine the effect of heat treatments on the nutritional quality and safety of rice bran (Amarasinghe *et al*., 2004). Therefore, the present study was aimed to produce a poultry feed, incorporated with rice bran. Rice bran was processed with different heat treatments and tested for nutrient quality and microbiological safety.

2. MATERIALS AND METHODS

2.1 Rice bran sample collection

Gonabaru paddy (traditional rice variety) and BG 358 (improved rice variety) were collected from Anuradhapura, Sri Lanka. Part of the Gonabaru and BG 358 paddy were parboiled, and parboiled rice bran was collected before the milling process. In parboiling, the clean paddy was soaked in cold water for 2-3 hours, put in parboiling equipment with fresh cold water, and boiled until it began to split. Then the paddy was dried on woven mats, cooled, and freshly milled (Mujahid *et al*., 2004). All parboiled and crude paddy from two rice varieties was milled from the local rice milling facility. Then, all rice bran samples were packed in polyethylene bags and stored in the refrigerator at -20 ºC (Samli, 2006).

2. 2 Heat treatments

Parboiling, dry heating, autoclaving and microwave heating were the four different heat treatments used in this study. In dry heating (hot air oven heating), crude rice bran was transferred into shallow pans and spread uniformly in a layer of about 0.5 cm thickness. Pans were then placed in a preheated oven at 80 ºC for 6 minutes (Gopinger *et al*., 2019).

Autoclaving was another method applied as heat treatment for rice bran samples. Rice bran was placed in a sealed polyethylene bag and heated in an autoclave at 121 ºC for 20 minutes (Irakli *et al*., 2021). In microwave heating, the microwave chamber was preheated at 100% power for three minutes, and rice bran was placed in a glass bowl covered with a glass lid with a small opening which was subjected to microwave heating at 100% power for two minutes (Kim *et al*., 2014). After the heat treatment, the sample was cooled to room temperature, and re-packed in sealed polyethylene bags separately until the preparation of poultry feed. Four replicates from crude, parboiled and other types of heat-treated rice bran of two varieties were prepared and kept separately inside the sealed polythene bags.

2.3 Preparation of poultry feed incorporating heat-treated rice bran

All the ingredients of the experimental feed for laying hens were mixed, and prepared the poultry feed separately. Other ingredients of the poultry feed formulation were same, except for the differently treated rice bran of the two varieties (Table 1). Five types of poultry feeds were prepared by incorporating the 200 g of crude or differently heat-treated rice bran of each rice variety. These were crude rice bran incorporated poultry feed (T1), parboiled rice bran incorporated poultry feed (T2), autoclaved rice bran incorporated poultry feed (T3), dry heated rice bran incorporated poultry feed (T4), and microwave heated rice bran incorporated poultry feed (T5) in four replicates of two rice varieties. Then, it was packed in polyethylene bags, sealed, and stored to evaluate the storage stability and nutritional quality at room temperature (Friedman, 2013).

Table 1: Ingredients of experimental feed for laying hens

Ingredients of experimental feed	Amount %
Corn	53.53
Rice bran	10.00
Full-fat soybean	9.23
Soybean meal	13.28
Gluten meal	2.00
Fish meal	1.00
Limestone	9.3
Mono calcium phosphate	0.95
Salt	0.22
$NaHCO_3$	0.10
Vitamin + mineral mix	0.25
Phytase	0.02

2.4 Nutritional value Analysis

The amount of crude protein, crude fiber and fat content were determined in each test samples. Nitrogen was determined as total Kjeldahl nitrogen by digestion of 1.0 g sample in 2 ml of concentrated sulfuric acid and subsequent nitrogen determination on a Kjeltec TM 8200 distiller (Fabian and Ju, 2011). The fat content was determined by Bligh and Dyer method. The crude fiber was determined as the weighed residue on a frittered-glass crucible, after sequential 5% sulphuric acid digestion, 5% sodium hydroxide digestion and ashing at 500 °C (Debi *et al*., 2019). Moisture was determined by the dry weight basis method; drying 5 g samples for 16 hours at 105 °C in a forced-air oven.

Dry matter (DM) was calculated using DM % = 100 – moisture % and the protein was estimated as following equation

$$\text{Crude protein (CP)} = \text{Nitrogen } \% \times 6.25$$

2. 5 Microbiological Analysis

In pour plate method, potato dextrose agar (PDA) medium acidified with tartaric acid (10%) was used to isolate the contaminated fungi. After sample inoculation, the cultures were incubated at 25 °C for 72 hours (Oliveira *et al*., 2011). Nutrient agar medium was used to quantify bacteria and after sample inoculation, the cultures were incubated at 35°C for 24 hours (Supriyati *et al*., 2015). The results were expressed as colony-forming units per gram (CFU/g) of the sample. All the samples were evaluated at 0, 2, and 4 weeks of storage.

2.6 Statistical analysis

The data were analyzed statistically using MINITAB software (Version 2016). Dunnett Method and 95% confidence were used to compare the mean difference between treatments and control. Tukey's method was used and 95.0% confidence level for testing the differences among treatment means.

3. RESULTS AND DISCUSSION

3. 1 Nutritional value analysis

Total protein, lipid, and dietary fibre contents were measured in the crude and heat-treated rice bran incorporated poultry feed in two rice varieties (V 01- BG 358 rice variety and V 02- Gonabaru rice variety).

It was revealed that crude rice bran of Gonabaru rice variety (V02) incorporated poultry feed was shown significantly high ($p<0.05$) total protein content than the other four differently heat-treated rice bran incorporated meal (T2, T3, T4, T5) (Fig. 1). In T3, T4 and T5, the heat treatment of the rice bran of two varieties were not differed significantly (Fig. 1).

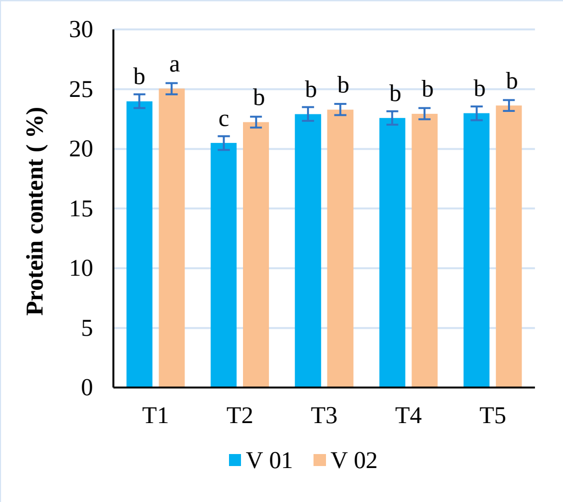

Figure 1: Percentage of protein content in rice bran of two rice varieties incorporated poultry feed in different treatments. Error bars denote the standard deviation. Means that do not share the same letter are significantly different at p< 0.05.

[T1- Control (Crude rice bran incorporated poultry feed), T2- Parboiled rice bran incorporated poultry feed, T3- Autoclaved rice bran incorporated poultry feed, T4-Dry heated rice bran incorporated poultry feed, T5-Microwave heated rice bran incorporated poultry feed. V 01- BG 358 rice variety and V 02- Gonabaru rice variety].

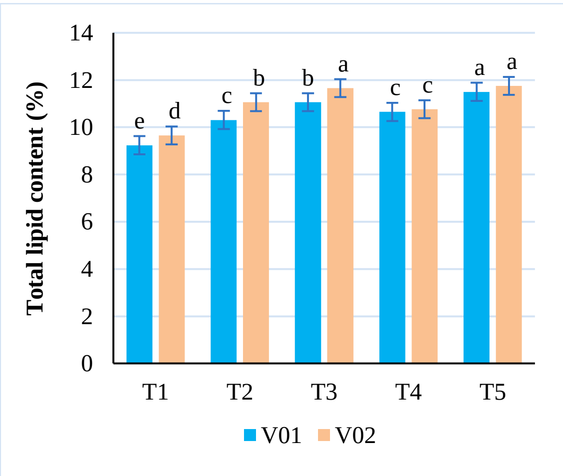

Figure 2: Percentage of total lipid content in rice bran of two rice varieties incorporated poultry feed in different treatments. Error bars denote the standard deviation. Means that do not share the same letter are significantly different at p< 0.05.

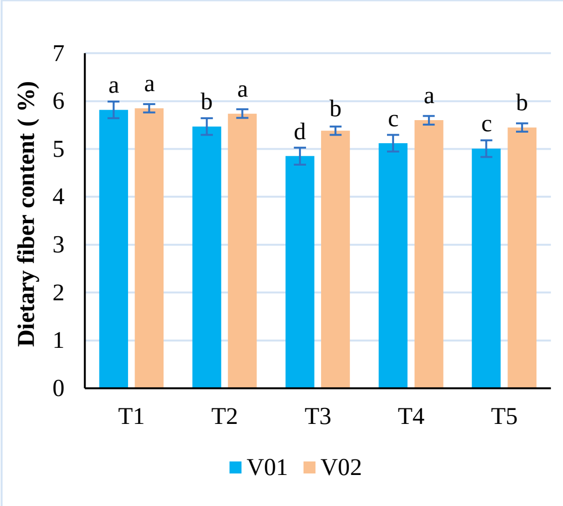

Figure 3: Percentage of dietary fibre content in rice bran of two rice varieties incorporated poultry feed in different heat treatments. Error bars denote the standard deviation. Means that do not share the same letter are significantly different at p< 0.05.

The total lipid content of poultry feed from two rice varieties revealed that four treatments (T2, T3, T4, T5) have significantly higher (p <0.05) lipid content than the control (T1). T5 of BG 358 (V 01) showed the highest lipid content and while crude rice bran incorporated feed (T1) showed the lowest. However, considering the Gonabaru rice variety (V 02), both T3 and T5 showed the highest lipid content at the 95% confidence level.

Endogenous lipase enzyme activates during milling, resulting in rapid deterioration of the oil due to hydrolytic and oxidative rancidity, rendering it unsuitable for consumption and also reducing its nutritional quality (Deniz *et al*., 2007; Amerasinghe *et al*., 2009). Therefore, the reduction of fat content in the control than the other treatments may be due to the increased action of the lipase enzyme that immediately breakdown the fat when separated from the rice kernel (Saunders, 1985; Lakkakula *et al*., 2004; Sharif *et al*., 2014).

The application of heat treatment differently (T2, T3, T4, T5) exhibited lower fibre content than the control (T1). Furthermore, the statistical

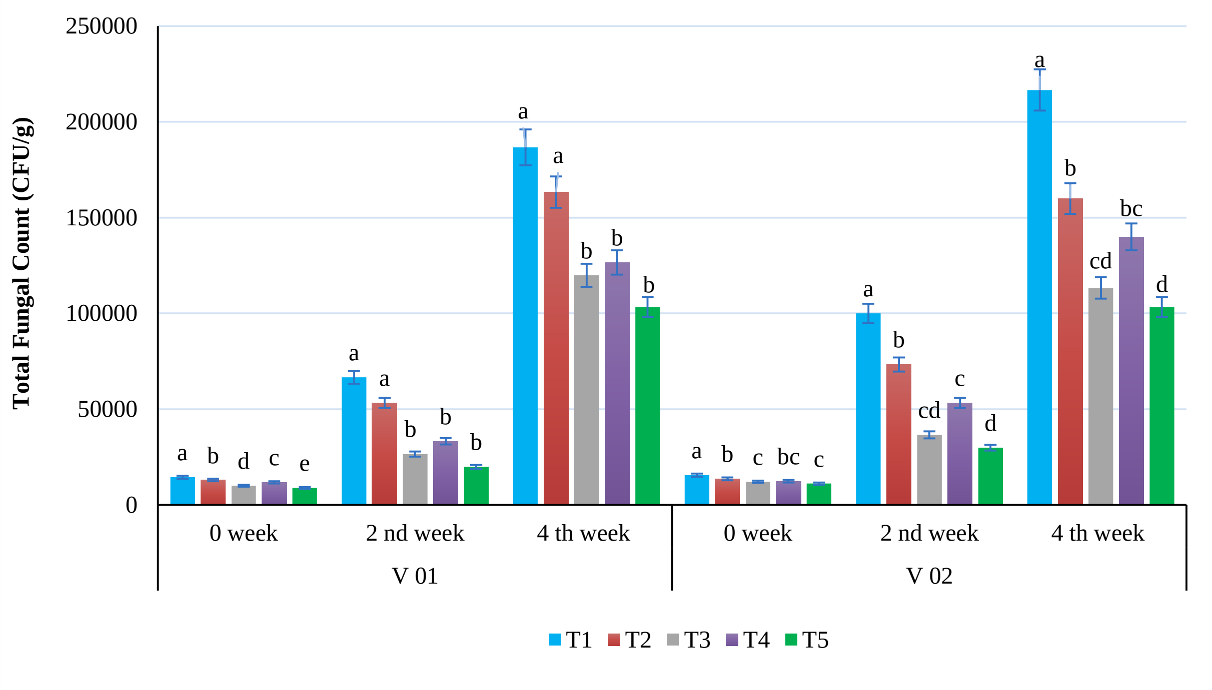

Figure II: Total fungal count of rice bran incorporated poultry feed with the time under different treatments. Error bars denote the standard deviation. Means that do not share a same letter are significantly different at $p < 0.05$. Significant difference denoted by letters separately in 2 rice varieties and the treatments.

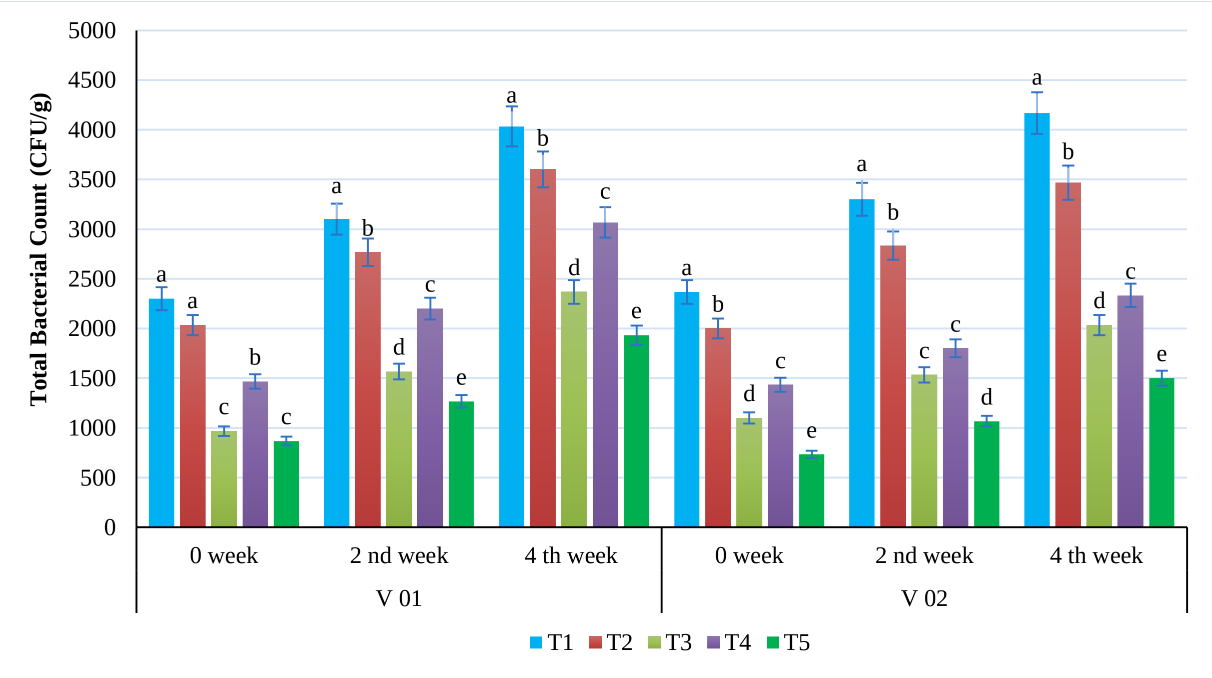

Figure 5: Total bacterial count of rice bran incorporated poultry feed with the time under different treatments. Error bars denote the standard deviation. Means that do not share a same letter are significantly different at $p < 0.05$. Significant difference denoted by letters separately in 2 rice varieties and the treatments.

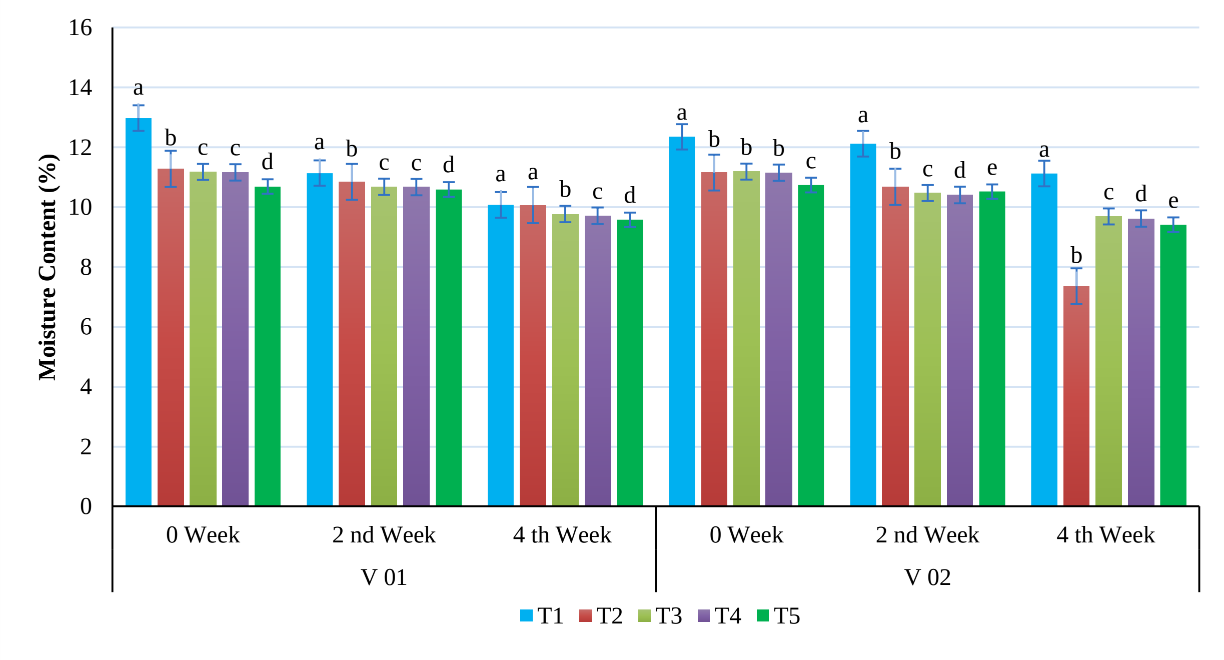

Figure 6: Moisture content of rice bran incorporated poultry feed with the time under different treatments. Error bars denote the standard deviation. Means that do not share a same letter are significantly different at p< 0.05. Significant difference denoted by letters separately in 2 rice varieties and the treatments.

analysis revealed that the dietary fibre content of T2, T3, T4, and T5 treatments were significantly different ($p<0.05$) from the control (T1) (Fig. 3). The high fibre content in rice bran limits the potential use of rice bran in poultry diets. Because it acts as an antinutritional factor that increases the passage rate in the gut and brings physicochemical changes in the ingesta due to its hydrophilic property (Yamin *et al*., 2020). A reduction in fiber content in feeds that were prepared by incorporating the heat-treated rice bran from both varieties revealed that heat processing can be used to reduce the fibre content.

3. 2 Microbiological Analysis

The total fungal count of rice bran from both BG 358 (V01) and Gonabaru (V02) incorporated poultry feeds revealed that four treatments (T2, T3, T4, T5) exhibited less fungal count than the control (T1) for all storage periods (Fig. 4). The heat processed rice bran incorporated poultry feed showed the reduction of fungal population count compared to crude rice bran for all measured time intervals. Furthermore, it was revealed that total fungal counts were increased with the time. In every tested storage period, the lowest fungal count has been shown by the T5 in both rice varieties bran incorporated poultry feed.

Statistical analysis revealed that the total bacterial count from both V01 and V02 variety rice bran incorporated poultry feeds, four treatments (T2, T3, T4 and T5) exhibited less bacterial count than the control (T1) for all the tested storage periods. Furthermore, T2, T3, T4 and T5 were significantly different from the control (T1) in the second and fourth week of the storage in V01. But in 0 weeks, T3, T4, and T5 were significantly different from the control (T1) at the 0.05 probability level except for the T2. In V02 rice bran incorporated poultry feed, four treatments (T2, T3, T4, T5) are significantly different from the control (T1) in all the storage periods. However, the lowest bacterial count has been shown by the microwave heated rice bran incorporated poultry feed (T5) in both rice varieties (Fig. 5).

3. 3 Chemical analysis

According to the statistical analysis of data, T2, T3, T4 and T5 were significantly different from the control (T1) at the 0.05 probability level in 0 and 2nd week of the storage in V01. But in the 4th week, T3, T4, and T5 were significantly different from the control (T1) except the T2. In V02 rice bran incorporated poultry feed, four treatments (T2, T3, T4 and T5) are significantly different from the control (T1) in all the storage periods. In 0 weeks, T2, T3, and T4 were not significantly different from each other (Fig. 6).
Besides avoiding bacterial and fungal growth, bran stability was favored by low moisture content (Ruan *et al*., 2015). The relationship between moisture content and fungal growth is in agreement with (Khazari *et al*., 2018), who stated that moisture and temperature are critical factors for fungal growth and mycotoxins production. This can be proven in the present study, when considering the total fungal count and moisture content in poultry feeds prepared by incorporating heat-treated rice bran.

4. CONCLUSION

Heat treatments used in the study were effective in lowering microorganisms present in the rice bran incorporated poultry feed. Although the protein content has been reduced in heat-treated rice bran incorporated poultry feeds, lipid content has been preserved. The heat treatments were effective in reducing the high fiber content which is considered as an antinutritional factor of rice bran. Among other heat treatments, microwave heat treatment is the most suitable method to stabilize the rice bran. Although the values are different the effect of the heat treatments on the nutritional quality and microbiological safety of rice bran incorporated poultry feed is affected in the same manner for two tested rice varieties.

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