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Nutritional upgrading of various feed ingredients through co-culture solid state fermentation

Çeşitli yem içerikleri besin değerlerinin birlikte kültür katı hal fermentasyonu kullanılarak artırılması

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Abstract: Objective: The purpose of the present study was to enhance nutritional qualities of various agricultural byproducts such as wheat bran, rice bran and rice polish through co-culture solid state fermentation (SSF) using Candida utilis and Rhizopus oligosporus for their better exploitation as feed ingredients.

Methods: Co-culture solid state fermentation (SSF) was carried at 30°C for 48 h by employing Candida utilis and Rhizopus oligosporus on various agricultural byproducts like wheat bran, rice bran and rice polish. After that the fermented agricultural byproducts were dried in hot air oven at 80°C and analyzed to compare with unfermented byproducts.

Results: The results of the proximate analysis showed that crude protein contents increased significantly \((p \leq 0.05)\) in all the fermented substrates with concurrent decrease in nitrogen free extract (NFE) contents. A significant reduction in anti nutritional content (phytic acid) was also observed in fermented products whereas values of mineral contents [calcium (Ca) and phosphorus (P)] were found high. Resultantly, high mineral contents improved Ca: P ratio in the fermented products. Shelf life study showed that the fermented substrates were nutritionally stable and no significant changes in nutritional values were observed up to 90 days.

Conclusion: All these results showed that the fermented substrates are nutritionally better and can be successfully exploited as animal feed for better growth of livestock.

Keywords: Feed ingredients, co-culture, fermentation, Rhizopus oligosporus, Candida utilis

Özet: Amaç: Bu çalışmanın amacı, buğday kepeği, pirinç kepeği, pirinç kabuğu gibi çeşitli tarımsal yan ürünlerin yem içeriği olarak kullanılarak Candida utilis ve Rhizopus oligosporus kullanılarak birlikte kültür katı hal fermentasyonu (SSF) ile besin değerlerinin artırılmasıdır.

Metod: Birlikte kültür katı hal fermentasyonu (SSF) 30°C de 48 s Candida utilis ve Rhizopus oligosporus kullanılarak buğday kepeği, pirinç kepeği, pirinç kabuğu gibi çeşitli tarımsal yan ürünler üzerinde uygulanmıştır. Daha sonra elde edilen yan ürünler sıcak hava firmında 80°C de kurutulmuşlar ve ferment ve olamanı ürünler ile karşılaştırarak sureti ile analiz edilmiştir.

Bulgular: Yaklaşık analiz sonuçlarına göre, ham protein içerikleri, tüm ferment ve substratları istatistiksel olarak anlamalı \((p \leq 0.05)\) şekilde yükselirken azot içermeyen ekstrakt (NFE) içerikleri belirgin şekilde düşmüştür. Fermente ürünlerde önemli bir anti besin bileşeni olan fitik asitin belirgin bir şekilde düştüğü gözlenenir mineral içeriklerinin (kalsiyum (Ca) ve Fosfor (P)) arttığı saptanmıştır. Sonuç olarak yüksek mineral içerikleri, fermente

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1 Introduction

Livestock play an important role in socio-economic development of any country having abundant sources of agricultural byproducts. These agricultural byproducts are the main ingredient of animal feed to provide energy and protein to the livestock for their growth. But due to relatively poor nutritional quality of these feed ingredients, the health conditions of most of the animals are not satisfactory. Among them, many animals are suffered in various diseases due to malnutrition. On the other hand, the requirement of protein and other food products for livestock are increasing day by day globally with increase in human population. In the current scenario, it is necessary to search new economical unconventional feed resources to obtain higher nutritional values. Utilization of unconventional resources for animal feed has been made possible by the process known as solid state fermentation (SSF) [1,2]. In solid state fermentation (SSF), microorganisms are cultivated on the agricultural byproducts to produce single cell protein and various other biological products. Various raw materials such as soybean and soybean meal [3,4], cottonseed meal [5] and some other agricultural byproducts [6,7] have been fermented to improve their nutritional values as well as to eliminating anti-nutritional factors. Solid state fermentation (SSF) not only improved nutritional values but also improve their sensory characteristics, thus representing a potential solution to the feed industry by providing nutritional rich feed ingredients to overcome the malnutrition in animals.

The aim of the present study was therefore to improve the nutritional qualities of various agricultural byproducts extensively used in feed formulations to make them more valuable for livestock exploitation by employing co-culture solid state fermentation process.

2 Materials and Methods

2.1 Substrate

Various agricultural byproducts such as wheat bran, rice bran and rice polish were purchased from local market and stored in a clean polythene bag at room temperature during the study period. These substrates were further cleaned to remove the impurities prior to use in solid state fermentation process.

2.2 Microorganisms

The fungus culture *Rhizopus oligosporus* and yeast *Candida utilis* strain were obtained from Microbiology Lab, Food & Biotechnology Research Centre (FBRC), Pakistan Council of Scientific and Industrial Research (PCSIR), Lahore, Pakistan.

2.3 Maintenance of microorganisms

The cultures of *Rhizopus oligosporus* and *Candida utilis* were grown on potato dextrose agar (Lab M., UK) by incubating the slants at 30°C for 4 days and then preserved the slants at 4°C in a refrigerator. The cultures were further shifted on freshly prepared slants after interval of 30 days to keep them revive during the entire period of study.

2.4 Inoculum preparation

The inoculums of *Rhizopus oligosporus* and *Candida utilis* were prepared by transferring a loopful culture of each strain into 50 mL inoculum medium consisted of 2% glucose, 0.4% potato starch and 0.2% di-ammonium phosphate in separated 250 ml conical flask. Thereafter, each inoculum medium was incubated at 120 rpm in an orbital shaker (SKIR-602L, Korea) at 30°C for 48 h. The pH of the inoculum medium was adjusted at 6.0 with 1 N HCl/NaOH prior to autoclaving the media at 121°C (15psi) for 15 min.

2.5 Process of solid state fermentation (SSF)

Six set of experiments were conducted to evaluate the changes in the nutrient profile of various agricultural byproducts such wheat bran, rice bran and rice polish by solid state fermentation (SSF).
2.5.1 Experiment-1

In experiment-1, 500 g of wheat bran having 45% moisture level was supplemented with 2.0% (w/w) diammonium phosphate. The moist wheat bran was inoculated with 48 h old inoculums of *Rhizopus oligosporus* and *Candida utilis*. After inoculation with 10% (w/v) of each culture, the processed wheat barn was incubated at 30°C for 48 h in perforated polyethylene bag.

2.5.2 Experiment-2

About 500 g of rice bran after maintaining at 35% moisture level was supplemented with 2.0% (w/w) diammonium phosphate. Thereafter rice bran was inoculated with 48 h old inoculums of *Rhizopus oligosporus* and *Candida utilis* with 10% (w/v) of each culture and incubated at 30°C for 48 h perforated polyethene bag.

2.5.3 Experiment-3

In experiment no. 3, about 500 g of rice polish moistened up to 45% moisture level was inoculated with 48 h old inoculums of *Rhizopus oligosporus* and *Candida utilis*. After inoculation with each culture (10%, w/v), the inoculated rice polish was incubated at 30°C for 48 h in perforated polyethylene bag.

2.5.4 Experiment-4

In experiment no. 4, about 500 g of properly mixed ingredients (wheat bran and rice polish) substrate in the ratio of 1:1 was supplemented 2.0% (w/w) diammonium phosphate. Thereafter mixed ingredient substrate after maintaining 45% moisture level was inoculated with 48 h old inoculums of *Rhizopus oligosporus* and *Candida utilis* and incubated the substrate at 30°C for 48 h in perforated polyethylene bag.

2.5.5 Experiment-5

In experiment no. 5, 500 g of mixed ingredient substrate (wheat bran and rice bran) after mixing in the ratio of 1:1:1 was supplemented with 2.0% (w/w) diammonium phosphate. Thereafter mixed ingredient substrate was inoculated with inoculum medium (10%, w/v) of each culture and incubated at 30°C for 48 h. The moisture level of substrate was maintained at 45% at the time of inoculation with each culture (10%, w/v). The inoculated substrate was incubated at similar conditions as mentioned in previous experiments.

2.5.6 Experiment-6

In this experiment, 500 g of mixed ingredients substrate (wheat bran, rice bran and rice polish) after mixing in the ratio of 1:1:1 was supplemented 2.0% (w/w) diammonium phosphate. Thereafter mixed ingredient substrate was inoculated with inoculum medium (10%, w/v) of each culture and incubated at 30°C for 48 h. The moisture level of substrate was maintained at 45% at the time of inoculation.

2.6 Analytical methods

Moisture, ash, crude protein (% Nitrogen x 6.25), fat contents (ether extract) and crude fiber contents of unfermented and fermented products were determined according to the approved method of AOAC [8]. Nitrogen free extract (NFE) content in each sample was calculated by difference. For pH determination, 1 g sample was dissolved in 10 mL distilled water and then centrifuged at 7000 rpm for 10 min and pH of the supernatant was measured with a digital pH meter (Jenway, UK). All the measurement was made in triplicates.

2.7 Determination of metabolizable energy content

Metabolizable energy (ME) was calculated according to the following formula:

\[
\text{ME (Kcal/g)} = \frac{(3.5\times\%CP) + (8.5\times\%CF) + (3.5\times\%NFE))}{100}
\]

Where, CP=Crude Protein; CF=Crude Fat; NFE=Nitrogen Free Extract (carbohydrate)

2.8 Anti-nutritional component

Anti-nutritional component (phytic acid) of unfermented and fermented products was determined according to the method of Young & Greaves [9]. As per method, 5 g sample was soaked in 200 mL of HCl solution (2%, v/v) for 2–3 h. Thereafter, the soaked sample was filtered through a double layered filter paper and added 10 mL of 0.30% ammonium thiocyanate solution containing 0.00195 g Iron/mL as an indicator into the 50 mL of filtrate. The volume was then made up to 167 mL with deionized water. This solution was titrated against 0.566% (w/v) iron chloride solution.
to develop slightly brownish yellow color which persisted for 5 min, as an indication of end point. The phytic acid content was estimated as given below formula.

\[ \text{Phytic acid contents(\%)} = X \times 1.19 \times 100 \]

Where \( X \) = titer value \times 0.00195 g; 1.19 is a factor

Estimation of inorganic minerals

For the estimation of inorganic minerals calcium (Ca) and phosphorus (P), ash from known quantity of dried sample was dissolved in 100 mL deionized water at ambient temperature. After proper mixing, the solution was filtered through 0.45 um syringe filter and then filtrate was used to estimate the inorganic minerals (Ca and P) in both unfermented and fermented samples.

### 2.9 Estimation of calcium concentration

Calcium concentration was determined spectrophotometrically using reagent kit prepared by PCSIR Labs Complex, Karachi, Pakistan. About 20 μl of sample solution was added to 2 mL of monoreagent containing methyl thymol blue as a color complex solution. The reaction mixture was allowed to stand at room temperate for 10 min and then absorbance was measured at 578 nm against blank. Inorganic phosphorus concentration was determined by comparing with the absorbance of a standard having known concentration.

### 2.10 Estimation of inorganic phosphorus concentration

Inorganic phosphorus was also estimated spectrophotometrically using a reagent kit obtained from PCSIR Labs Complex, Karachi, Pakistan. According to the procedure, 10μl of filtered solution was added to 1 mL of ammonium molybdate solution. The reaction mixture was allowed to stand at room temperate for 10 min and then absorbance was measured at 340 nm against blank. Inorganic phosphorus concentration was determined by comparing with the absorbance of a standard having known concentration.

### 2.11 Statistical analysis

Analysis of variance (ANOVA) was performed by considering variation in proximate composition of various agricultural byproducts during solid state fermentation. The difference among the means values was determined through Tukey’s test at 5% level of significance using SPSS Statistics 19 software.

### 3 Results and Discussion

The improvement in nutritive value of feed ingredients through solid state fermentation (SSF) is emerging tool now days and representing a potential solution to feed industry regarding the nutritive quality of feed. In present study various agro-industrial by-products such as wheat bran, rice bran and rice polish widely used in animal feed were employed in co-culture solid state fermentation (SSF) process to evaluate the changes in nutritive profiles of these bye-products in separate and mixed forms as shown in Table 1. Significant differences (\( p < 0.05 \)) were observed in the values of various components during proximate analysis of unfermented and fermented sub-

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**Table 1: Nutritional profile of various agricultural byproducts before and after solid state fermentation by employing co-culture of Rhizopus oligosporus and Candida utilis.**

| Substrate | Dry matter (%)±SD | Crude protein (%)±SD | Crude fat (%)±SD | Crude fiber (%)±SD | Ash content (%)±SD | Nitrogen free extract (%)±SD | Metabolizable energy (Kcal/g)±SD |
|-----------|-------------------|----------------------|------------------|-------------------|-------------------|-----------------------------|-------------------------------|
| UWB       | 91.7±0.35a        | 14.2±0.27a           | 2.1±0.15b        | 10.5±0.18b        | 4.5±0.27a         | 59.8±0.56i                  | 2.80±0.03c                    |
| URB       | 94.1±0.30a        | 10.3±0.15b           | 8.8±0.08a        | 12.2±0.28a        | 11.2±0.06b        | 51.4±0.34h                  | 2.92±0.01f                    |
| URP       | 94.3±0.26ab       | 16.3±0.23d           | 15.4±0.08b       | 10.5±0.11b        | 10.7±0.27a        | 41.2±0.30b                  | 3.3±0.01h                     |
| FWB       | 90.0±0.10a        | 21.0±0.27b           | 1.3±0.08a        | 12.0±0.26a        | 6.7±0.19b         | 48.8±0.36h                  | 2.56±0.01a                    |
| FRB       | 88.2±0.32a        | 15.8±0.22b           | 8.3±0.26a        | 12.3±0.26a        | 13.0±0.04a        | 38.6±0.16d                  | 2.62±0.02b                    |
| FRP       | 92.8±0.25c        | 19.5±0.26d           | 13.0±0.13b       | 9.3±0.11c         | 12.5±0.17h        | 38.4±0.70b                   | 3.13±0.02f                    |
| FWB+FRB   | 93.3±0.15c        | 19.1±0.06e           | 6.7±0.15c        | 12.5±0.24a        | 8.4±0.21b         | 46.6±0.69c                  | 2.87±0.01c                    |
| FWB+RPB   | 92.0±0.18e        | 23.7±0.26c           | 8.9±0.07f        | 13.2±0.24f        | 8.6±0.25d         | 37.4±0.92c                   | 2.90±0.02g                    |
| FWB+FRB+FRP | 89.4±0.27a      | 20.3±0.13d           | 10.1±0.07a       | 13.9±0.08a        | 9.1±0.07f         | 35.8±0.23b                   | 2.83±0.01c                    |

UWB: Unfermented wheat bran; URB: Unfermented rice bran; URP: Unfermented rice polish; FWB: Fermented wheat bran; FRB: Fermented rice bran; FRP: Fermented rice polish; Each value is mean of three replicate and the values followed by different letters within column are significantly different by Tukey’s test (\( p<0.05 \)) ±standard deviation (SD).
strates. Crude protein values of fermented substrates were noted significantly higher than those obtained from unfermented substrates. These findings indicated that crude protein contents increased due to the microbial growth in the fermented substrates. Similar observations in protein enrichment of agricultural by-products have also been reported by various investigators [7,10,11]. Decreases in values of nitrogen free extract (NFE) were obtained in fermented materials as compared to unfermented material. The reduction in NFE may be attributed to the efficient utilization of carbohydrates (which constitute the NFE) by microbes to produced microbial protein and different types of enzymes which are proteinaceous in nature. A similar work in which R. oligosporus along with Trichoderma hazarium and Aspergillus niger enhanced protein contents by exploiting carbohydrates available in the agricultural bye-products has reported earlier [12]. A reduction in NFE contents due to its biotransformation into protein rich material during SSF has also been reported in another investigation [6]. Similarly a slight reduction was observed in crude fat contents in fermented materials which might happened due to utilization of available fat contents for microbial growth. These results are in accordance with earlier reported findings in which a reduction in fat contents of rice husk was observed during solid state fermentation by Trichoderma viride [13]. In general fermentation reduced crude fiber contents especially when substrates are fermented with fungi because they posses ability to produce cellulases that degraded lignocellulosic fiber [14]. However, in present study a slight increase in crude fiber contents was observed in all the fermented materials. It might be possible due to utilization of easily available digestible carbohydrate contents present in substrates by growing microbes as reported earlier [15].

Energy content of substrates is another important parameter to explore its application in feed formulation. An insignificant inconsistency in the values of energy contents among various unfermented and fermented substrates were observed as shown in Table 1. The results indicated that such variation is attributed due to change in nutritional profiles of various substrates by fermentation. Similar pattern of inconsistency of energy contents were obtained during biodegradation of rice husk to improve its nutritional values by Trichoderma viride [13].

Phytic acid is known as an anti-nutrient mainly due to its ability to bind essential dietary minerals including calcium, iron and zinc, as well as protein and starch, consequently reduce their bioavailability [16,17]. In present study, a phytic acid content decreased considerably in all the fermented substrates. On the other hand, the biodegradation of phytate increased calcium to phosphorous (Ca:P) ratio by increasing calcium and phosphorous contents in the fermented substrates (Table 2). Similar findings regarding increase in minerals contents were observed by some other investigators while working on ripening of cheese and biodegradation of rice husk [13,18]. However, the reduction in phytate contents in present study may be connected to the release of phytase enzyme from R. oligosporus during fermentation process. Various fungi have already been exploited for phytase production by using agricultural bye-products [19,20]. All these findings indicate that microbes were able to degrade anti-nutritional component (phytic acid) which resultantly improve bioavailability of mineral contents, prevent protein phytate complex, leaving more protein available for digestion and absorption in the fermented products.

| Substrate   | Phytic acid content (%)±SD | Calcium(Ca) (%)±SD | Phosphorous (P) (%)±SD | Ca/P     |
|-------------|--------------------------|-------------------|-----------------------|----------|
| UWB         | 5.19±0.06a               | 0.36±0.02a        | 0.79±0.02a            | 0.46±0.01b |
| URB         | 9.59±0.27h               | 0.14±0.01a        | 1.21±0.06e            | 0.11±0.01f |
| URP         | 9.22±0.09h               | 0.48±0.03h        | 0.90±0.07h            | 0.53±0.08e |
| FWB         | 2.61±0.08a               | 0.89±0.07a        | 1.55±0.06e            | 0.57±0.02b |
| FRB         | 6.70±0.26a               | 0.33±0.01h        | 1.75±0.09e            | 0.19±0.01f |
| FRP         | 5.43±0.07h               | 0.67±0.08e        | 1.16±0.04e            | 0.57±0.05e |
| FWB+FRB     | 6.60±0.23a               | 0.72±0.05a        | 1.23±0.07e            | 0.59±0.07b |
| FWB+FRP     | 6.35±0.18a               | 0.82±0.04e        | 1.42±0.11d            | 0.58±0.07b |
| FWB+FRB+FRP | 6.85±0.16a               | 0.73±0.05e        | 1.28±0.03e            | 0.57±0.05b |

UWB: Unfermented wheat bran; URB: Unfermented rice bran; URP: Unfermented rice polish; FWB: Fermented wheat bran; FRB: Fermented rice bran; FRP: Fermented rice polish; Each value is mean of three replicate and the values followed by different letters within column are significantly different by Tukey’s test ($p$≤0.05) ±standard deviation (SD).
A suitable longer shelf life of fermented feed ingredients is another important parameter to explore its application in livestock for the production of fresh milk, meet, eggs and broilers. Therefore, the shelf life study was conducted by storing various fermented agricultural by-products at room temperature up to 90 days and a complete proximate analysis of each fermented substrate was conducted after every 30 days. No significant changes were observed in nutritional values of the fermented agricultural by-products during storage at room temperature up to 90 days (Table 3–5). These results clearly indicate that the fermented agricultural by-products contained stable nutrient components which are completely suitable for a longer period as feed supplements.

The results of present study clearly indicate that protein contents of various agricultural byproducts increased significantly through co-culture solid state fermentation (SSF) by Candida utilis and Rhizopus oligosporus. A considerable reduction in anti-nutritional ingredient (phytic acid) along with improvement in mineral contents in all the combination of substrates was also observed. All these findings suggest that the fermented agricultural by-products are the good source of feed and feed formulation to overcome the protein deficiency in livestock.

### Table 3: Nutritional profile of various fermented agricultural byproducts after storage of 30 days at room temperature.

| Substrate | Dry matter (%)±SD | Crude protein (%)±SD | Crude fat (%)±SD | Crude fiber (%)±SD | Ash content (%)±SD | Nitrogen free extract (%)±SD | Metabolizable energy (Kcal/g)±SD |
|-----------|-------------------|----------------------|------------------|-------------------|-------------------|-----------------------------|-------------------------------|
| FWB       | 96.89±1.22b       | 22.08±0.56^ab       | 1.35±0.10a       | 11.57±0.26^a      | 6.73±0.10a        | 55.15±1.52^a                | 2.82±0.03^a                   |
| FRB       | 96.15±0.42a       | 15.61±0.32b         | 7.76±0.29a       | 12.34±0.61b       | 14.14±0.49^g      | 46.30±0.47^d               | 2.83±0.02^a                   |
| FRP       | 95.38±0.83^a      | 20.19±0.46^bc       | 14.01±0.88^f     | 10.63±0.79^g      | 11.92±0.56^a      | 38.62±0.94^f               | 3.25±0.06^e                   |
| WB+RB     | 96.40±0.83^a      | 19.97±0.36^a        | 5.96±0.23^b      | 11.85±0.36^a      | 7.95±0.19^a       | 50.67±0.14^f               | 2.98±0.03^e                   |
| WB+RP     | 94.83±0.72^a      | 24.32±0.68^bcd      | 9.53±0.61^d      | 12.71±0.43^f      | 8.37±0.11^e        | 39.89±1.33^f               | 3.06±0.07^f                   |
| WB+RB+RP  | 94.53±0.78^a      | 21.08±0.94^bcd      | 9.83±0.60^d      | 13.56±0.39^f      | 9.99±0.20^a        | 40.08±0.61^a               | 2.98±0.01^c                   |

FWB: Fermented wheat bran; FRB: Fermented rice bran; FRP: Fermented rice polish; Each value is mean of three replicate and the values followed by different letters within column are significantly different by Tukey’s test (p≤0.05)±standard deviation(SD).

### Table 4: Nutritional profile of various fermented agricultural byproducts after storage of 60 days at room temperature.

| Substrate | Dry matter (%)±SD | Crude protein (%)±SD | Crude fat (%)±SD | Crude fiber (%)±SD | Ash content (%)±SD | Nitrogen free extract (%)±SD | Metabolizable energy (Kcal/g)±SD |
|-----------|-------------------|----------------------|------------------|-------------------|-------------------|-----------------------------|-------------------------------|
| FWB       | 95.11±0.83^a      | 21.54±0.66^e         | 1.41±0.05^a      | 11.38±0.12^a      | 6.87±0.16^a       | 53.91±0.14^i               | 2.76±0.03^a                   |
| FRB       | 94.40±0.56^a      | 15.03±0.15^a         | 7.95±0.10^a      | 12.42±0.06^a      | 13.71±0.23^f      | 45.28±0.26^d               | 2.79±0.01^a                   |
| FRP       | 96.02±0.49^ab     | 19.99±0.22^k         | 13.85±0.09^a    | 11.01±0.32^a      | 11.79±0.22^e      | 39.38±0.17^g               | 3.26±0.02^e                   |
| FWB+FRB   | 95.94±0.43^abc    | 19.15±0.25^lm        | 6.23±0.25^c     | 11.93±0.28^b      | 8.18±0.03^b       | 50.45±0.13^f               | 2.97±0.01^lm                   |
| FWB+FRP   | 95.01±0.30^a      | 23.15±0.62^d         | 8.68±0.18^a     | 12.51±0.15^e      | 8.78±0.27^g       | 41.89±0.86^c               | 3.01±0.01^d                   |
| FWB+FRB+FRP | 94.15±0.73^a   | 19.98±0.28^b         | 9.95±0.23^a     | 13.80±0.21^d      | 10.32±0.24^e      | 40.10±0.62^b               | 2.95±0.03^ab                   |

FWB: Fermented wheat bran; FRB: Fermented rice bran; FRP: Fermented rice polish; Each value is mean of three replicate and the values followed by different letters within column are significantly different by Tukey’s test (p≤0.05)±standard deviation(SD).

### Table 5: Nutritional profile of various fermented agricultural byproducts after storage of 90 days at room temperature.

| Substrate | Dry matter (%)±SD | Crude protein (%)±SD | Crude fat (%)±SD | Crude fiber (%)±SD | Ash content (%)±SD | Nitrogen free extract (%)±SD | Metabolizable energy (Kcal/g)±SD |
|-----------|-------------------|----------------------|------------------|-------------------|-------------------|-----------------------------|-------------------------------|
| FWB       | 94.74±0.50^a      | 22.41±0.28^a         | 1.33±0.10^a      | 10.30±0.51^a      | 6.89±0.18^a       | 53.80±0.86^a               | 2.78±0.03^a                   |
| FRB       | 95.22±0.12^a      | 16.15±0.36^b         | 7.67±0.37^a      | 12.01±0.30^de     | 13.83±0.27^e      | 45.56±1.23^m               | 2.81±0.01^b                   |
| FRP       | 96.58±0.57^ab     | 21.21±0.47^f         | 13.40±0.49^h     | 11.16±0.27^h      | 11.22±0.36^e      | 39.59±1.58^h               | 3.27±0.01^e                   |
| FWB+FRB   | 97.08±0.45^abc    | 18.86±0.46^bc        | 6.34±0.39^h     | 12.02±0.37^de     | 7.95±0.20^c       | 51.91±0.64^h               | 3.01±0.02^f                   |
| FWB+FRP   | 95.78±0.51^a      | 22.96±0.35^a         | 8.70±0.40^a     | 12.71±0.57^de     | 8.79±0.28^f       | 42.62±0.80^b               | 3.03±0.02^f                   |
| FWB+FRB+FRP | 95.84±0.54^a   | 19.17±0.49^h         | 9.97±0.38^a     | 13.47±0.34^de     | 10.33±0.25^e      | 42.90±0.79^bc              | 3.02±0.02^f                   |

FWB, Fermented wheat bran; FRB, Fermented rice bran; FRP, Fermented rice polish; Each value is mean of three replicate and the values followed by different letters within column are significantly different by Tukey’s test (p≤0.05)±standard deviation(SD).
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