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The potency of *Pediococcus pentosaceus* incubated with *Moringa oleifera* in fermentation process to increase nutrient content of rice bran

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Abstract. This study aimed to improve the nutritional quality of rice bran by using *Pediococcus pentosaceus* incubated with *Moringa oleifera* extract (*MOE*) extract in rice bran fermentation. The treatments were divided into 7 treatments and 3 replications, as follows: P0: control; P1: 1% *P. pentosaceus*); P2: (1% *P. pentosaceus* + 1% *MOE*); P3: (1% *P. pentosaceus* + 2% *MOE*); P4: (2% *P. pentosaceus*; P5: (2% *P. pentosaceus* + 1% *MOE*); P6: (2% *P. pentosaceus* + 2% *MOE*). *MOE* was added to probiotics according to the dose of treatment and then incubated at 37ºC for 24 hours. Furthermore, synbiotic had been used to ferment rice bran for 5 days under anaerobic conditions. The results indicate that there was a significantly different ($p < 0.05$) in the group treatments and group control on the content of extract ether, crude protein, nitrogen-free extract, crude fiber, and metabolizable energy. The use of synbiotics in the treatment showed an increase in crude protein by 16.76%, a decrease in crude fiber by 23.35, a decrease in extract ether by 19.86%, NFE 9.47%, and an increase in metabolizable energy from 3057.41 to 3153.08 kcal/kg. Based on the results of this study, it could be concluded that the use of *P. pentosaceus* incubated with *M. oleifera* extract could be used to improve the nutritional quality of rice bran.

1. Introduction

Probiotics are live microbial, non-pathogenic, when consumed by humans or livestock, have a positive effect by improving its intestinal microbial balance. Several kinds of probiotics used for livestock include *Lactobacillus casei* WB 315 [1], *Bifidobacterium sp*, *L. acidophilus*, and *L. casei* [2], [3], *Bifidobacterium spp.* and *Lactobacillus casei* [4], *Pediococcus acidilactici* [5], *Lactobacillus salivarius* and *Pediococcus pentosaceus* [6]. Probiotics can be used single or mixed, can be given through drinking water or feed. The common fermenting bacteria are species of *Pediococcus*, *Lactobacillus*, *Streptococcus*, *Micrococcus*, *Leuconostoc*, and *Bacillus* [7].

Prebiotics are a source of food ingredients that can be used by probiotics for the host health [8]. *Moringa oleifera* leaves contain a source of protein, polyphenols [9]. Polyphenols are associated with

antioxidant activities [10] and anti-inflammatory activity [11]. *Moringa oleifera* leaves also contain dietary fiber (total soluble and insoluble fiber, oligosaccharides, and resistant starch) [12]. Insoluble dietary fiber is a substance that is insoluble in water, is bulky, and lowering the food passage rate through the intestinal tract [13]. The content of polysaccharides in *Moringa oleifera* is 75.11% and had a molecular weight of 304,700 g/mol [14]. The polysaccharide can affect the increase of microbial composition in the intestinal tract of experimental animals, can produce short-chain fatty acid and lactic acid. This indicates that *Moringa oleifera* can be used as a prebiotic property [15], [16]. This study aims to prove the effect of *Moringa oleifera* extracts on the growth of *Pediococcus pentosaceus* to increase the nutrient content of fermented bran

2. Material and methods

2.1 Preparation of probiotic culture

The bacteria *Pediococcus pentosaceus* stock culture was stored at -80°C in MRS broth (Oxoid), adding by glycerol 20% (v/v). *P.pentosaceus* probiotic culture was mixed to MRS broth sterile and incubated at 37 °C for 24 hours. The culture was grow on de Man Rogosa Sharpe agar (Oxoid) at 37 °C for 24 h in incubator.

2.2 Fermentation process

Prepare synbiotic cultures of *Pediococcus pentosaceus* and *Moringa oleifera* extract according to the treatment. P0: control (without probiotic and without *Moringa oleifera* extract*);* P1: 1% *Pediococcus pentosaceus*; P2: 1% *Pediococcus pentosaceus* + 1% *Moringa oleifera* extract); P3: 1% *Pediococcus pentosaceus* + 2% *Moringa oleifera* extract; P4: 2% *Pediococcus pentosaceus*; P5: 2% *Pediococcus pentosaceus* + 1% *Moringa oleifera* extract; P6:2 % *Pediococcus pentosaceus* + 2% *Moringa* oleifera extract), and then the synbiotic was incubated for 24 hours. *Pediococcus pentosaceus* isolates that have been incubated with Moringa oleifera extract are then sprayed on a 100 grams rice bran for each treatment, incubated at anaerobic conditions for 5 days. After the fermentation process ends, then the plastic was opened and the bran is aerated for further proximate analysis.

2.3 Statistical analysis

All the data results were described as the mean ± standard deviation. The all data analized by using a statistical software system $p<0.05$ was described a significantly different.

3. Result and discussion

The result of nutrient content of rice bran fermented by proximate analysis in this study showed in Table 1.

Table 1. Nutrient content of rice bran fermented by synbiotic for 5 days.

	Crude Protein (CP)	Extract Ether (EE)	Crude Fiber (CF)	Nitrogen Free Extract (NFE)	Metabolizable Energy (ME)
P0	$13.72^{a} \pm 0.24$	$9.92^{e} \pm 0.01$	$17.47^{b} \pm 0.60$	$50.14^{a} \pm 0.83$	$3057.41^{a} \pm 20.97$
P1	$14.02^{b} \pm 0.30$	$8.16^{b} \pm 0.01$	$15.50^{ab} \pm 1.33$	$53.59^{b} \pm 1.65$	$3062.79^{ab} \pm 46.84$
P2	$14.47^{c} \pm 0.08$	9.20e± 0.01	$14.04^{a} \pm 1.79$	$53.70^{b} \pm 1.73$	$3153.08^{b} \pm 61.56$
P3	$14.62^{c} \pm 0.01$	$8.34^{c} \pm 0.01$	$13.77^{a} \pm 1.73$	$54.89^{b} \pm 1.74$	$3139.30^{ab} \pm 59.18$
P4	$15.23^{d} \pm 0.06$	$8.60^{d} \pm 0.01$	$13.39^{a} \pm 1.28$	$53.94^{b} \pm 1.35$	$3143.08^{ab} \pm 44.41$
P5	$16.02^{e} \pm 0.14$	$7.95^{a} \pm 0.01$	$14.02^{a} \pm 0.75$	$53.49^{b} \pm 0.81$	$3107.15^{ab} \pm 25.88$
P6	$15.23^{d} \pm 0.76$	$8.15^{b} \pm 0.01$	$13.84^{a} \pm 1.68$	$54.64^{b} \pm 1.84$	$3137.45^{ab} \pm 58.35$

The results of the statistical analysis of the crude protein content of rice bran fermented for 5 days, showed that there were significantly different in the group treatments ($p<0.05$) (listed in Table 1, Fig

1). The average crude protein content of fermented bran was 13.72% to 16.02%. The lowest of crude protein value was found in the control (P0) of 13.72%, which was a significant difference from all treatments P1, P2, P3, P4, P5, and P6. The highest crude protein content of rice bran fermented was in treatment P5 (16.02%), namely the use of 2% *Pediococcus pentosaceus* + 1% *Moringa oleifera* extract, which was a significant difference from all treatments P0, P1, P2, P3, P4 and P6. The increase crude protein was 16.76% compared to the control. An increase in crude protein content indicates the growth of *Pediococcus pentosaceus* bacteria during the fermentation process. These results were in line with Vijayalakshmi [17] who indicated that the use of *Moringa oleifera* have a positive effect on the growth of *Lactobacillus rhamnosus*. The results of this study are also in line with the research of previous study [18], [19].

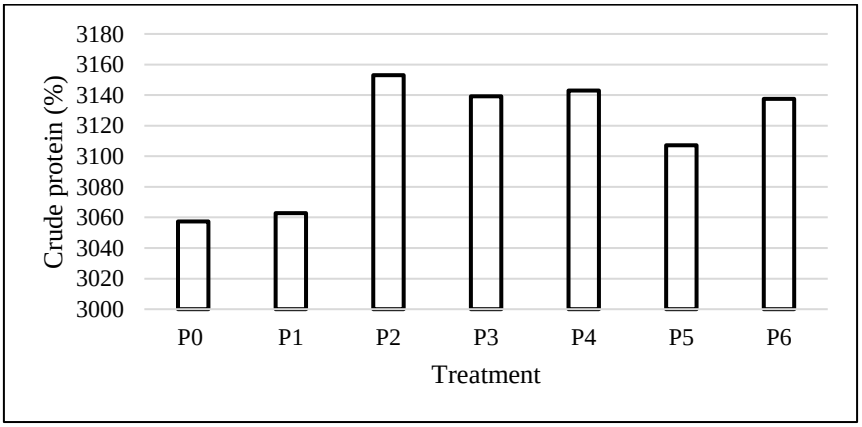

Figure 1. Crude protein content of fermented rice bran by *P.pentosaceus* + *M.oleifera* extract.

The results of the statistical analysis of extract ether content in rice bran fermented for 5 days, indicated that there was significantly different in the group treatments ($p<0.05$) (listed in Table 1, Fig 2). The average extract ether content of fermented rice bran was 7.95% to 9.92%. The highest extract ether content was found in the control treatment (9.92%), which was not significantly different from the P2 treatment, namely the use of 1 % *Pediococcus pentosaceus* + 1% *Moringa oleifera* extract, but there were significant differences with treatment P1, P3, P4, P5, and P6. The lowest extract ether content was found in treatment P5 (7.95%), namely the use of 2% *Pediococcus pentosaceus* + 1% *Moringa oleifera* extract, which was different from all treatments P0, P1, P2, P3, P4, and P6. The decrease in ether extract was 19.86% when compared to the control. Decreasing of extract ether content indicated the growth of *Pediococcus pentosaceus* bacteria during the fermentation process. These results study similar by El-Sayed [20], that supplemented by 0.5% *Moringa oleifera* leaves powder affected increasing of growth lactic acid bacteria and probiotic culture.

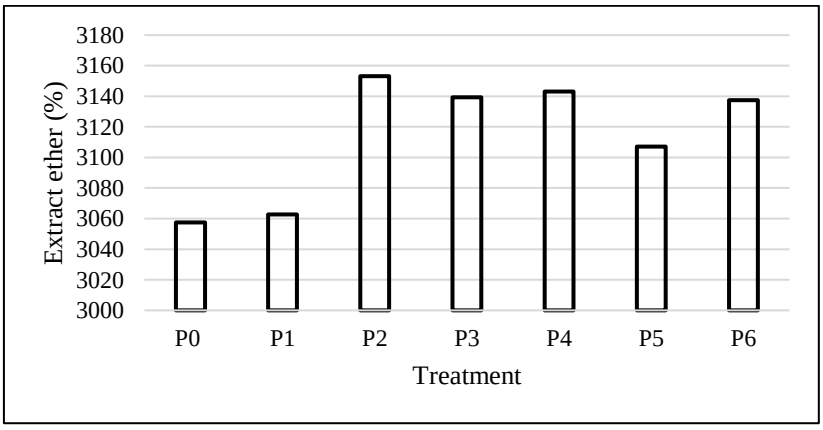

Figure 2. Extract ether content of fermented rice bran by *P.pentosaceus* + *M.oleifera* extract.

The results of the statistical analysis of the crude fiber content in rice bran fermented for 5 days, indicated that there was significantly different in the group treatments ($p<0.05$) (listed in Table 1, Fig 3). The average crude fiber content in fermented rice bran was 13.84% to 17.47%. The highest crude fiber content was found in the control treatment (17.47%), which was different from all treatments P1, P2, P3, P4, P5, and P6. The lowest crude fiber value was found in group P6 (13.84%), the treatment using 2% *Pediococcus pentosaceus* + 2% *Moringa oleifera* extract. P6 treatment was significantly different from control treatment, but not significantly different from treatment P1, P2, P3, P4, P5. The decrease in crude fiber was 23.35% when compared to the control. Decreasing of crude fiber content indicates the growth of *Pediococcus pentosaceus* bacteria during the fermentation process. These results study similar by El-Sayed [20], that supplemented by *Moringa oleifera* affected increasing of growth lactic acid bacteria and probiotic culture.

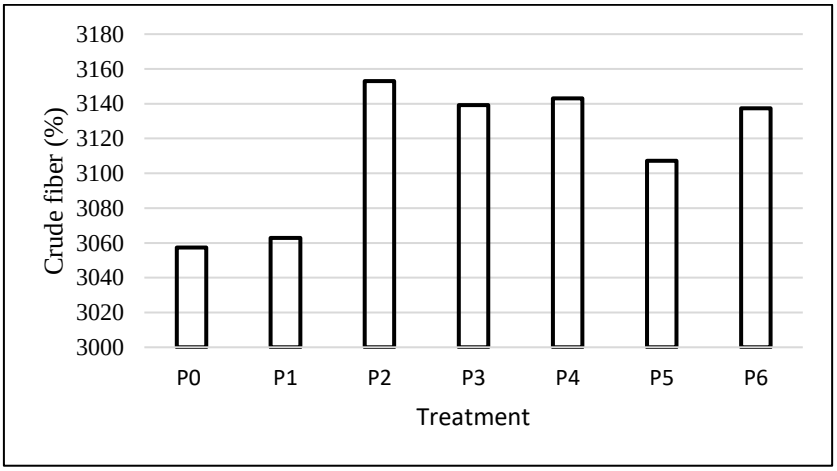

Figure 3. Crude fiber content of fermented rice bran by *P.pentosaceus* + *M.oleifera* extract

The results of statistical analysis of the NFE content in rice bran fermented for 5 days, indicated that there was significantly different in the group treatments ($p<0.05$) (listed in Table 1, Fig 4). The average NFE content in the treatment was 50.14% to 54.89%. The lowest NFE content was found in control and significantly different with all treatments, while the highest NFE content was found in treatment 1% *Pediococcus pentosaceus* + 2% extract *Moringa oleifera* (P3) which was not different from treatment P1, P2, P4, P5 and P6. The increase in NFE was 9.47% compared to the control. The growth of isolate in this the fermentation process is influenced by the availability of nutrient in the substrate. Nitrogen free extract content was the result of the qualitative changes caused by the activities of microorganism. In this research showed that the growth of *Pediococcus pentosaceus* utilizing nutrient in the *Moringa oleifera* extract.

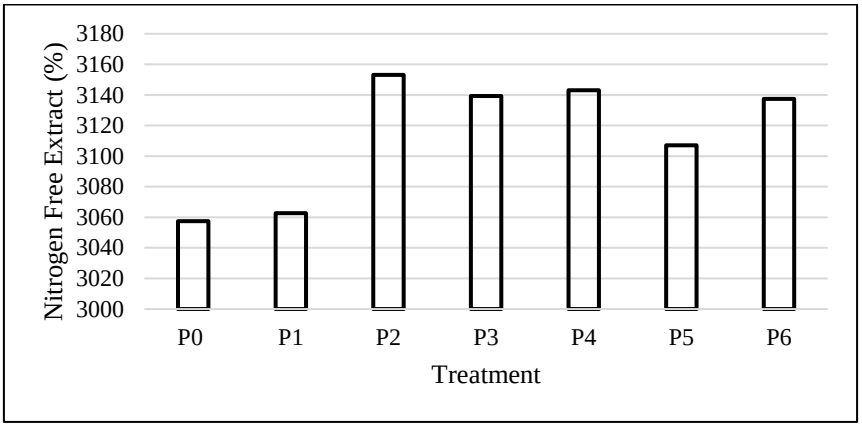

Figure 4. NFE content of fermented rice bran by *P.pentosaceus* + *M.oleifera* extract.

The results of the statistical analysis of ME content in rice bran fermented for 5 days showed that there was significantly different in the group treatments ($p<0.05$) (listed in Table 1, Fig.5). The average ME content in the treatment was 3057.41 kcal/kg to 3153.08 kcal/kg. The lowest ME content in control was significantly different from P2 treatment, but not different from the other treatments P1, P3, P4, P5, and P6. The highest ME content was found in the treatment of 1% *Pediococcus pentosaceus* + 1% extract *Moringa oleifera* (P2), but did not significant differences from treatment P1, P3, P4, P5, and P6. The ME content in the fermented rice bran showed that more higher than without fermenting rice bran by synbiotic. This research indicates that the availability of biological values from fermented rice bran [21]. The results of the research that the addition synbiotic contain prebiotic *M.oleifera* extract could affect the growth of *Pediococcus pentosaceus* probiotics.

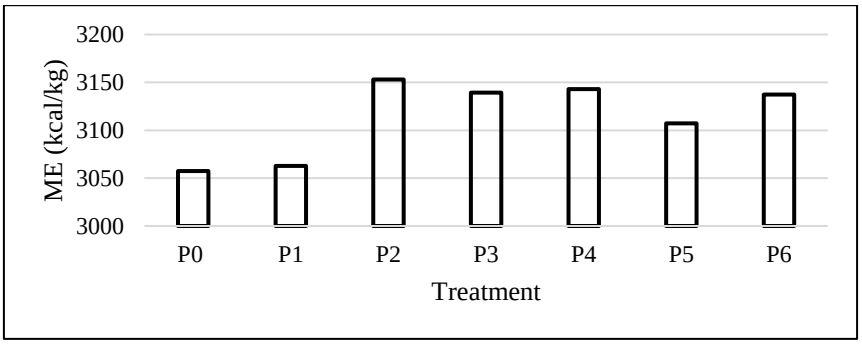

Figure 5. ME content of fermented rice bran by *P.pentosaceus* + *M.oleifera* extract.

4. Conclusion

The use of synbiotic of 2% *Pediococcus pentosaceus* + 1% *Moringa oleifera* extract can increase crude protein content, nitrogen free extract and metabolizable energy and reduce crude fiber, extract ether.

5. References

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