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Nutritive Value of Fermented Banana *Pseudo* Stem (*Musa spp*) and Rice Bran by *Saccharomyces cerevisiae*

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***Abstract* – Banana pseudo stem was generally utilized by the farmers as their pig feed in the rural areas of Cambodia after harvested the fruit. It was mixed with rice bran plus cooked rice or/and kitchen wastes prior to feeding. However, it has very low nutritive value and higher fiber and water content, so the possibility of methods in improvement of its nutritive value should take-into account for its better effectively utilization for the household pig production. This experiment was done to determine the optimum treatment by application of *Saccharomyces cerevisiae* as initial growth promotor in different fermentation times to improve nutritive-value of banana pseudo stem. The design was followed by treatments with completely randomized design (CRD) and 4 replicates. The experiment (I) was done on the fermentation of banana pseudo stem and rice bran with *Saccharomyces cerevisiae* solution in aerobic condition (in foam boxes) at the different fermentation times of 0, 1, 3, 5 and 7 days, the samples from each period were taken for chemical analysis of DM, Ash, CP, TP and CF. As the result, DM, CP, Ash and TP of fermented banana pseudo stem were increased significantly, following to the treatments but quite decreased CF content from 31.65% to 15.55%. In addition, it was noticed that the best time of fermentation of BS40RB60 in this study was 7 days which could significantly improve nutritive value, especially true protein from 9.54-14.51% and crude fiber content from 18.07-13.99% ($p<0.001$). In addition, for the experiment (II) was tested on the comparison between two treatments of fermented banana pseudo stem and rice bran with and without *Saccharomyces cerevisiae* solution at the different fermentation times. As the result, the DM and Ash of (FBSW) was higher than (FBS), but the CP and TP content were significantly lower, following to the times of fermentation. It was found that treatment with addition of *Saccharomyces cerevisiae* solution was higher in nutritive values, compare to the treatment without any addition of *Saccharomyces cerevisiae* solution.**

***Keywords* – Banana *Pseudo* Stem, Rice Bran, *Saccharomyces cerevisiae*, Fermentation Time and Aerobic Condition.**

I. Introduction

Forty percent of banana plants was considered as waste in the field to decay and about 60-80 tons/ha/year of banana pseudo stem was produced [1]. However, it has been known as low nutritive value and high-water content. Banana stem contains high water content of 93.4% but has low nutritive value of CP 6.5% and lipid 1.5% in DM basis [2]. Banana pseudo-stem has high ash content up to 14%, typically constituted by K 33.4%, Ca 7.5%, Mg 4.3%, Si 2.7 and P 2.2%, but lower lignin content of 12% compared with other plants [3]. Moreover, it had lignocellulose of 60% – 85% and cellulose of 50% [4] and about 50% cellulose, over 17% lignin and 4% ash but its leaves had about 26 % cellulose, 17% hemicellulose, and 25% lignin [5]. Crude fiber of inner portion of banana pseudo stem is about 20% and 40% in the outer portion as its ash content is from 14-30% and the fat ranging from 18 - 22% of outer and inner portion respectively [6]. However, banana stem could be improved by making a silage together with other foliage such as taro foliage or with yeast as growth promoter in order to improve protein content, feed intake and live weight gain as well [7], [8], [9]. Ensiling can also render some previously unpalatable products useful to livestock by changing the chemical nature of the feed [10]. Crops ensiled with moisture of 85% may result in effluent dry matter losses up to 10%, resulting in the loss of valuable nutrients because water and soluble nutrients accumulate at the bottom of the silo as silage effluent whereas crops wilted to about 70% moisture produce little effluent [11]. Ensiling was performed to achieve a target silage DM concentration of between 25 to 30% [12]. Dry matter of rice bran was quite high about 89% [13], so it could be potentially used to increase the dry matter of banana stem before ensiling with application yeast as growth promoter.

Saccharomyces cerevisiae is generally used broadly for fermentation of many organic and fiber material to produce foods for human such as bread, wine, sake and beer. However, it could be used in the diet of pigs for improvement of the intestinal environment, growth rate and digestibility and also prevention of colonization of pathogenic bacteria in small intestine [14]. *Saccharomyces cerevisiae* can grow very well with the present of carbon from the molasses and of other nitrogen sources, from urea. In order to increase biomass of *Saccharomyces cerevisiae*, the substrate must have enough soluble carbohydrate and nitrogen for the growth of their cells. The use of sugarcane blackstrap molasses and yeast extract at a C and N ratio of 10 gave the highest biomass of yeast. *Saccharomyces cerevisiae* usually can use some of the amino acids in molasses, but addition of nitrogen sources from urea is required. Both nitrogen source, nitrogen concentration and C/N ratio affect biomass concentration of the yeast [15]. However, any toxics can decrease yeast performance by inhibiting growth [8]. The feed with supplementation of live yeast may result in increasing greater absorption of the nutrient by small intestine [16]. *Saccharomyces cerevisiae* could be used to ferment cassava pulp and root to improve the nutrition [17]. Fermented rice bran with yeast could improve crude protein up to 17.8% and reduction of crude fiber, NDF and ADF of 10.3%, 33.4 and 13.7%, respectively [18]. Replacing ensiled banana pseudo stem with taro by feeding rice bran of 40% could improve live weight gain and feed conversion of duck [19]. The banana pseudo stems were traditionally fed the pigs together with rice bran by farmers mostly in rural areas of Cambodia. The purpose of this study was to evaluate the nutritional value of various ratios of rice bran and banana stem through fermentation with *Saccharomyces cerevisiae*. Through these research results, the farmers can use fermented rice bran and banana stem to feed pigs under farm conditions.

II. Experiment I

A. *Location*

The experiment was carried out at laboratory office of the Center for Livestock and Agriculture Development (CelAgrid), located in Phnom Penh, Cambodia.

B. *Experimental Design*

The experiment was a factorial design with 4 treatments in completely randomize design (CRD) with four replicates. Four treatments were 100% banana pseudo stem in fresh basic (BS100RB0), 80% banana pseudo stem plus 20% rice bran in fresh basic (BS80RB20), 60% banana pseudo stem plus 40% rice bran in fresh basic (BS60RB40) and 40% banana pseudo stem plus 60% rice bran in fresh basic (BS40RB60), respectively. The formulation below was applied for all duration of fermentation.

Table 1. Formulation of fermentation of banana stem and rice bran in fresh basis

Composition	BS100 RB0	BS80 RB20	BS60 RB40	BS40 RB60
Banana stem (g)	100	80	60	40
Rice bran (g)	0	20	40	60
Urea (g)	0.8	1.79	2.78	3.77
S. cerevisiae solution (ml)	3 ml	3 ml	3 ml	3 ml

C. *Material Arrangement*

Banana stalks were bought from the farm nearby after harvested their fruits and then, chopped into small piece by electronic chopping machine. However, they were also peeled out some outside harder parts about 3-4 layers, after chopped. Chopped banana stalk was used at the same day to mix with other substrates. Rice bran was purchased from bigger scale of rice milling station that could produce the quality one with CP content of 10.5%. On other hand, *Saccharomyces cerevisiae* was bought from Feed Active Dry Yeast of ICFOOD Company and the molasses was bought from Cambodia agriculture development organization while the urea with 46 % of nitrogen content was purchased from the local market and tap water was used. The solution was composed by 0.5g *Saccharomyces cerevisiae*, 35g molasses, 63.5g water and 1.5g urea and then it was incubated in the room temperature for 24 hours before applied [20].

➢ *Banana Pseudo Stem Fermentation*

Chopped banana pseudo stems were replaced immediately by different levels of rice bran and urea, accordingly at the same day of chopping. It was noticed that the yeast solution was added in the same amount for all treatments (table 1). The completely mixed banana pseudo stem was incubated in foam boxes under the aerobic condition. The incubation time was arranged in the distinguished days of 0, 1, 3, 5 and 7 days, respectively.

D. *Chemical Analyses*

The banana pseudo stem silage was measurement of dry matter (DM), organic matter (OM), crude protein (CP) and true protein (TP) for 0, 1, 3, 5 and 7 days after fermentation. The dry matters (DM) of samples were determined by temperature - limited oven at a constant temperature of 60^{o}C for 24h and organic matter (OM) = (100-ash) and CP (N* 6.25) was determined by kjeldahl digestion with sulphuric acid followed by distillation [21] methods and true protein (TP) was done by putting 2 g of the fresh sample into a 125ml Erlenmeyer flask with 50 ml of distilled water, allowed to stand for 30 minutes, after which 10ml of 10% TCA (trichloroacetic acid) were added and allowed to stand for a further 20-30 minutes. The suspension was then filtered through Whatman #4 paper by gravity. The filtrate was discarded, and the remaining filter paper and suspended substrate transferred to a kjeldahl flask for standard estimation of total N.

E. *Statistical Analyses*

Data were analyzed by General Linear Model Repeated Measures of the SPSS software. The model is:

$$Y = mean + treatment + time + e$$

Least Significant Difference (LSD) test was used to compare difference between treatment at p value<0.05.

III. Result of Experiment I

The chemical composition of each ingredients was analyzed for dry matter (DM), Ash, crude protein (CP) and crude fiber (CF). DM contents of fresh banana pseudo-stem, rice bran and molasses of this study were approximately 7.02, 88.44 and 85.91%, respectively. The CP of banana pseudo stem, rice bran and molasses were 6.42, 10.50 and 5.25% DM, respectively (table 2).

Table 2. Chemical composition of each substrate in DM basic

Composition	DM; (%)	Ash; (%)	CP; (%)	CF; (%)
Banana stalk	7.02	1.52	6.42	32.64
Rice bran	88.44	10.03	10.50	21.5
Molasses	85.91	3.62	5.25	-
S. cerevisiae	-	-	-	-
Urea	-	-	46*6.25	-

➢ *Nutritive Value of different Rice Bran and Banana Pseudo Stem Ratios*

The nutritive value of different rice bran and banana pseudo stem ratios was presented in table 3. There were significant different in DM, Ash, CF, CP and TP content of all treatments (p<0.001). The DM, Ash, CP and TP were all

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increased following to level of replacing rice bran, while the CF content was reduced significantly (p<0.001). It was noticed that the highest true protein content of 11.50 (table 3) by replacement of 60% rice bran and 40% chopped banana pseudo stem and at the fermentation time of 7 days.

In this study, to evaluate the nutritional value of each treatment according to fermentation time. Samples were taken at times of 0, 1, 3, 5 and 7 days after fermentation. The results on nutritive values were shown in figure 1-5.

The DM content of all treatments were not significant different by fermentation time. The DM content of BS100RB0 was lowest. The DM of BS100RB0 at 0, 1, 3, 5 and 7 days were 7.60, 7.94, 8.18, 6.93 and 6.60%, respectively. While DM content of BS40RB60 was highest, and arranged from 52.38, 56.13, 52.04, 55.51 and 54.58 at 0, 1, 3, 5, and 7 days, respectively.

The Ash, CF, CP and TP content of treatment were significant different (p<0.001). The DM was not affected by fermentation time, but ash, CP, CF and TP content were significantly affected by fermentation time (figure 2, 3, 4 and 5). Highest TP content was at 7 days of fermentation time.

Table 3. Chemical composition of fermented banana stem at the different treatments

Treatment	Mean	95 % Confidence Interval Lower bound	95 % Confidence Interval Upper bound	Std-Error	p-value
DM, %					
BS100RB0	7.46^{d}	6.59	8.32	0.398	0.001
BS80RB20	22.70^{c}	21.84	23.57		
BS60RB40	38.17^{b}	37.30	39.04		
BS40RB60	54.19^{a}	53.32	55.06		
Ash, %					
BS100RB0	1.06^{d}	0.90	1.22	0.073	0.001
BS80RB20	2.64^{c}	2.48	2.80		
BS60RB40	4.56^{b}	4.40	4.71		
BS40RB60	6.09^{a}	5.93	6.25		
CF, %					
BS100RB0	31.65^{a}	31.06	32.23	0.268	0.001
BS80RB20	20.02^{b}	19.44	20.61		
BS60RB40	17.49^{c}	16.91	18.07		
BS40RB60	15.55^{d}	14.96	16.13		
CP, %					
BS100RB0	11.30^{c}	10.72	11.87	0.264	0.001
BS80RB20	15.42^{b}	14.84	15.99		
BS60RB40	17.23^{a}	16.65	17.80		
BS40RB60	17.63^{a}	17.06	18.21		
TP, %					
BS100RB0	9.09^{c}	8.61	9.57	0.220	0.001
BS80RB20	10.28^{b}	9.8	10.76		
BS60RB40	10.64^{ab}	10.16	11.12		
BS40RB60	11.50^{a}	11.02	11.98		

abcd: Mean values within column with different superscript letters are different at p<0.05

DM: Dry matter, CP: Crude protein, TP: True protein, CF: Crude fiber.

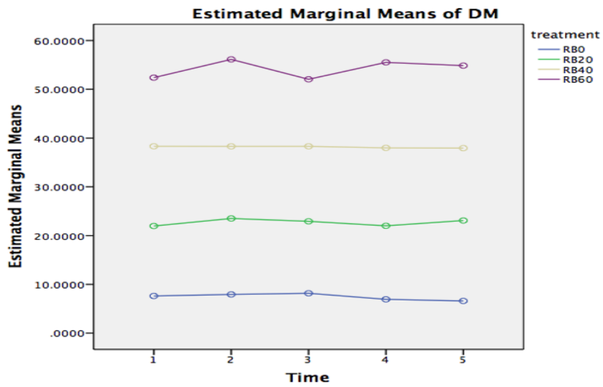

Fig. 1. Effect of fermented times at each treatment on DM

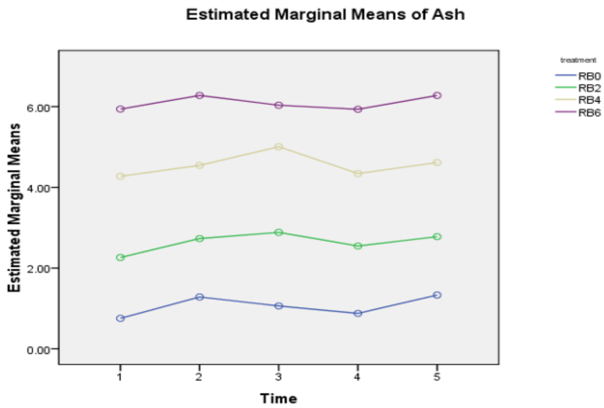

Fig. 2. Effect of fermented times at each treatment on ash

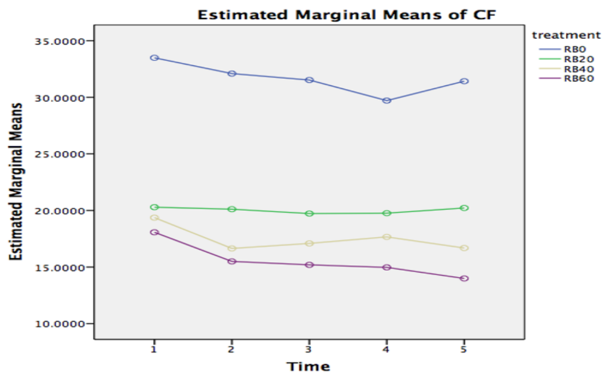

Fig. 3. Effect of fermented times at each treatment on CF

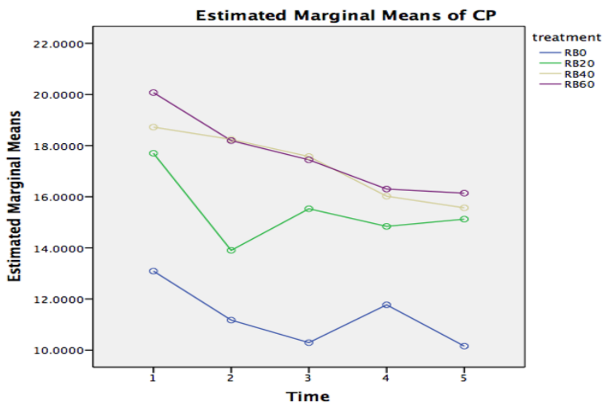

Fig. 4. Effect of fermented times at each treatment on CP

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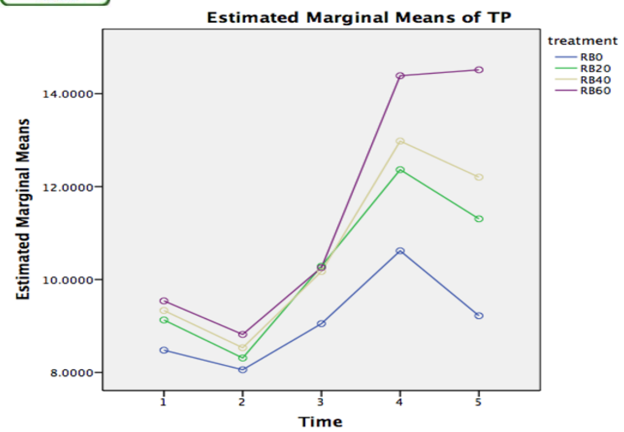

Fig. 5. Effect of fermented times at each treatment on TP
Notice: Fermented times was 0, 1, 3, 5 and 7 days.

➢ *Discussion of Experiment I*

CP, CF and ash content of fresh banana pseudo stem of this study was 6.42, 32.64 and 1.5%, respectively, while CP, EE, CF, Ash, NDF, ADF, hemicellulose, cellulose, lignin and tannin of banana stalk in DM basic were 7.2, 1.8, 31.5, 21.4, 67.2, 45.3, 21.9, 35.9, 9.4 and 0.74%, respectively [22]. The DM of banana stalk was up to 9% [23] and only average 6.5% [24] which was similar to this study of 7.02%. However, the varieties of banana, fertility of the soil and harvesting time were also affected on nutritive value of their stalk as well [25]. In this research, the DM content of the substrate were increased from 7.46-54.19% by increasing level of rice bran up to 60% in fresh basic ($p<0.001$), but it was not influenced by delaying the time of fermentation. This was because the originally effect of high DM content of rice bran. It was clearly that after adding rice bran, water content of substrate was reduce, and crude protein content of substrate was improved. Thus, rice bran could be primarily applied to increase the nutritive value of the banana pseudo stem. When long-time ensiling until 160 days resulted in a reduction in DM concentration in corn silages and with inoculation of lactic acid bacteria (LAB) resulted in higher concentrations of lactic acid and acetic acid in corn silages as well [26]. Lactic acid bacteria (LAB) grow most rapidly at temperatures between 27 and 38°C. Below 27°C, LAB growth is slower, but most fermentations should be complete between 7 to 10 days at these temperatures [27]. This study found that, there was no any change on DM content after 7 days of fermentation, but the times of fermentation was very influenced on CP and TP content. The CP and TP content were also increased up to 17.63 and 11.50%, respectively and CF content went down from 31.65-15.55% while increasing the level of rice bran up to 60%. In addition, within each treatment, CP content was decreased, whereas TP content was increased after 5 days of fermentation. After 7 days of fermentation (70:30 mixture of cassava pulp and rice bran) with urea, DAP and yeast, the level of true protein reached to 12.5% in DM when urea levels were applied in 2% of the substrate DM [28]. However, DM, CP, Ash and CF content of ensiled banana pseudo stem for 14 days without any addition of substrates were 12.20, 3.12, 11.60 and 36.60%, respectively [29] but DM, CP and Ash content of ensiled banana pseudo stem were 5.90, 4.90 and 3.10%, respectively [8]. It could be noticed that, there would be no any positive changes on nutritive value of banana silage but color, if no any additive substrates of carbon and nitrogen sources with application of yeast as starter. The nutritive value of banana pseudo stem of DM, CP, Ash, fat and carbohydrate including color could also be affected by drying temperature and timing [30] but freeze-drying was better than heat pump drying [1]. However, CP content was not significant change for banana pseudo stem silage from the first to 40th day of fermentation, but it began to increase from 50th day of ensiling. In addition, acetic acid levels were increased during fermentation and remained constant between days 20 and 30 [23]. Although silage fermentation was mainly due to homofermentative lactic acid bacteria, a small amount of acetic acid was also present due to heterofermentative lactic acid bacteria [31]. This rapid rise in the acetic acid concentration observed after 30 days of fermentation implied that homogeneous lactic acid fermentation had changed to heterogeneous lactic acid fermentation.

IV. Experiment II

This test was made to compare between two treatments of fermented banana pseudo stem with (FBS) and without *Saccharomyces cerevisiae* (FBSW) at different fermentation times (table 4). It means that the best treatment from experiment I above was selected to compare with another one without any addition of urea and *Saccharomyces cerevisiae* solution. Thus, there were two treatments of fermented banana stem with and without *Saccharomyces cerevisiae* (FBS and FBSW) at different fermentation times of 0, 1, 3, 5 and 7 days in completely randomized design (CRD) with 4 replicates.

➢ *Treatments:*

FBS: Fermented banana stem 40% and rice bran 60% (fresh basic), added urea 3.77g and *Saccharomyces cerevisiae* solution 3ml. FBSW: Fermented banana stem 40% and rice bran 60% (fresh basic) without *Saccharomyces cerevisiae* solution. Fermentation times**:** 0, 1, 3, 5 and 7 days. Statistical analysis and model were followed to the experiment I above.

V. Result of Experiment II

Chemical composition of banana stem and rice bran were analyzed for DM, Ash, CP and CF (table 4).

Table 4. Chemical composition of banana stem and rice bran (DM basic)

Composition	DM (%)	Ash (%)	CP (%)	CF (%)
Banana stem	6.52	1.68	6.58	31.16
Rice bran	88.73	13.13	10.50	20.61

DM content of FBSW started to be higher after five days of fermentation. It was also significantly different of both treatments and fermentation times ($p<0.001$). This was due to no aerobically microbial population for sugar metabolization of banana stem and rice bran as they could not grow well without any supplementation of carbon and nitrogen sources. In addition, organic matter (OM), crude protein (CP) and true protein (TP) were higher for FBS than another one (p<0.001). As result, nutritive value of banana stem and rice bran fermented by *Saccharomyces cerevisiae* as a starter with enough substrates, mainly carbon and nitrogen sources from molasses and urea were improved, accordingly.

Table 5. Chemical composition of fermented banana stem at the different treatments

Treatment	Mean	95% Confidence Interval Lower bound	95% Confidence Interval Upper bound	Std-Error	p-value
DM, %					
FBS	54.19	35.52	54.86	0.275	0.001
FBSW	59.89	59.22	60.57		
Ash, %					
FBS	6.09	6.01	6.17	0.033	0.001
FBSW	9.60	9.52	9.68		
CP, %					
FBS	17.63	17.34	17.92	0.119	0.001
FBSW	9.80	9.52	10.10		
TP, %					
FBS	11.50	11.20	11.81	0.124	0.001
FBSW	7.28	6.98	7.59		

FBS: Fermented banana stem 40%, rice bran 60% (fresh basic), urea 3.77 g with *Saccharomyces cerevisiae* solution 3 ml.
FBSW: Fermented banana stem 40% and rice bran 60% (fresh basic) without *Saccharomyces cerevisiae* solution.

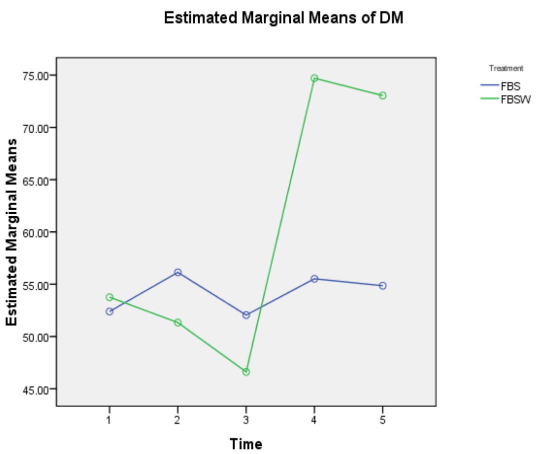

Fig. 6. Effect of fermented times at each treatment on DM

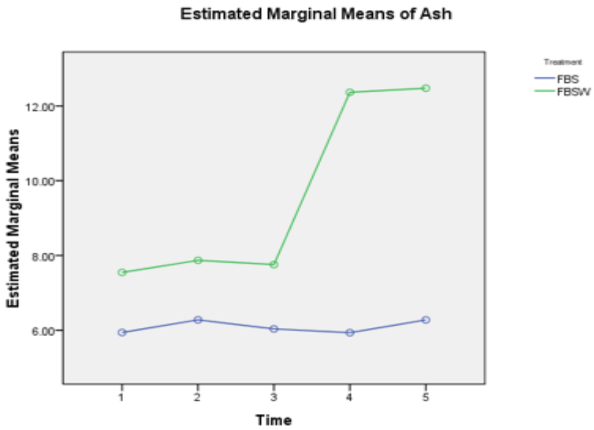

Fig. 7. Effect of fermented times at each treatment on ash

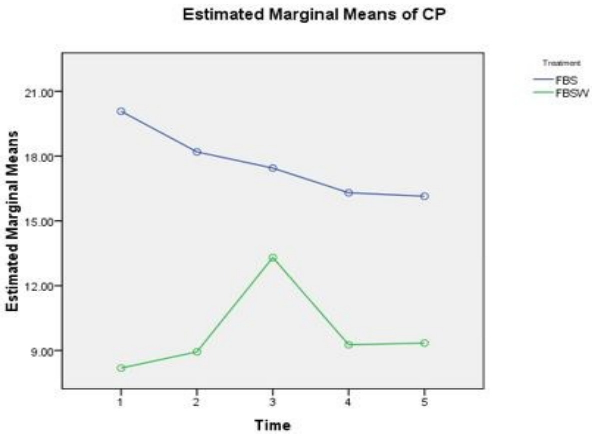

Fig. 8. Effect of fermented times at each treatment on CP

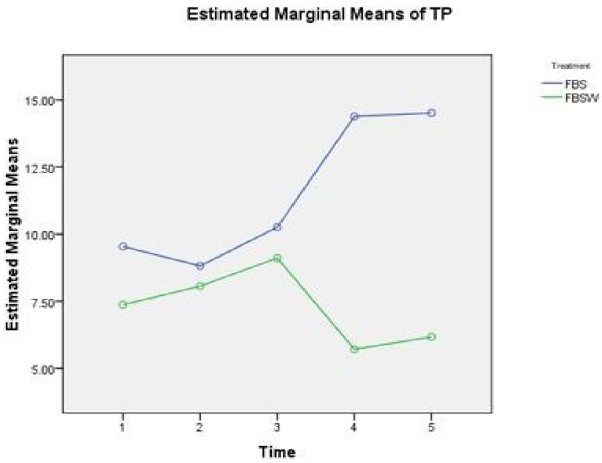

Fig. 9. Effect of fermented times at each treatment on TP
Notice: Fermented times were 0, 1, 3, 5 and 7 days.

- *Discussion of Experiment II*

Saccharomyces cerevisiae is one of the organisms that has been used for the production of single cell protein, it would produce good nutritional values of food and feed without toxic compounds [32]. Yeast single-cell proteins played a greater role in the evolution of aquaculture diets. With their excellent nutrient profiles, single-cell proteins have been added to aquaculture diets as partial replacement for fishmeal [33], [34]. This study stated that, application of

Saccharomyces cerevisiae solution as starter with supplementation of some substrates of urea as carbon and nitrogen sources and replacement of rice bran with banana stem at different level for increasing of DM could improve more nutritive value of the banana stem silage, mainly CP and TP content, compared to the one which was used only rice bran without any additive substances. This increasing of true protein was because the growth of biomass production of cellular *Saccharomyces cerevisiae*. Application of C/N ratio and molasses concentration as substrates could increase *Saccharomyces cerevisiae* biomass production [20]. Yeasts are continually challenged by stressful environments due to lack of oxygen, undesirable nutritional composition, especially limited amounts of nitrogen [35]. *Saccharomyces cerevisiae* fermentation products could improve microbial population that contributed to greater rumen fermentation efficiency by stimulating the growth and activity of fiber-digesting bacteria, increasing fiber digestion, increasing microbial protein synthesis [36]. Concentrations of *Saccharomyces cerevisiae* in the corn silage also increased after 7 days of aerobic exposure [37]. In addition, inoculant-treated silages with multiple strains of *Lactobacillus plantarum and Enterococcus faecium* had larger populations of LAB than the uninoculated controls (7.1×10^9 vs 2.3×10^9 cfu/g silage DM) and whole-crop barley ensiled at approximately 30% DM contained higher concentrations of soluble sugars and lactic acid [38].

The dry matter of treatment without application of *Saccharomyces cerevisiae* as a starter, but replacement of 60% rice bran with banana pseudo stem was higher after 5 days of ensiling, but CP and TP contents were lower, compared to the treatment with use of *Saccharomyces cerevisiae* solution as a starter. Chopped forage is still metabolically active and respires at ensiling stage while oxygen is available. Plant tissue respiration is the primary driver for removing oxygen from the silage and producing heat, although respiration by aerobic microorganisms can contribute [39].

VI. Conclusion

Application of *Saccharomyces cerevisiae* as starter and fermentation time were significantly affected the nutritive values of banana pseudo stem silage. This was positive option to improve the nutritive value of banana pseudo stem silage as feed resource for household pig production. Further research is required to define more appropriate techniques to improve the quality of banana pseudo stem as animal feed that could contribute to alleviation of production cost for household pigs' production.

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