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Research Paper

The Change of Nutrients Rations Quality of Feed Fermented with Different Moisture Content

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Abstract
The change of nutrients on rations made from local raw materials was evaluated after being fermented in various moisture contents. The experiment used completely randomized design comprise of 5 treatments and 3 repetitions. The treatments were P0 (fermentation without moisture), P1, P2, P3 and P4 (fermentation in 40%, 50%, 60% and 70% moisture content). Variables observed were total microbe, total acid, acidity (pH) and the change of dried matter content, organic matter, crude protein, crude lipid, crude fiber and Nitrogen-free extract. The result showed that there are statistically significance differences ($P<0.05$) between local raw materials rations fermented in different moisture contents towards variables observed. Local raw materials rations fermented in 50% moisture content exhibited good nutrients quality indicated by increased in dried matter content, organic matter, crude protein, Nitrogen-free extract, total microbe and total acid while crude lipid, crude fiber and acidity (pH) showed a decreased.

Keywords
fermentation, nutrient change, moisture content, local raw material rations

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1. INTRODUCTION

Rations is an important factor in supporting duck broiler farms. The cost for rations approximately 70% of total production budget in an intensive animal husbandry. Rations cost can be reduced by optimizing local raw materials obtained from agricultural and industrial wastes. Naturally, wastes from agriculture and industry has not been optimally utilized nevertheless one can get it easily. The drawback issues of wastes utilization as rations are highly crude fiber content, low digestible nutrients and the presence of anti-nutrients substance. Various waste processing can be carried out prior used as ration such as fermentation.

Fermentation is a process of storing substrates in anaerobic or aerobic conditions with embedded microbes in it. Fermentation process would reduce crude fiber content, increase digestible value and diminish anti-nutrients substance of local raw materials hence can be used as rations (Pamungkas, 2011). Inoculant widely used with the ability to hydrolyze starch in fermentation chamber is Saccharomyces cerevisiae contain in yeast. Saccharomyces cerevisiae is anaerobic facultative, which can live in both aerobic and anaerobic systems and able to break up glucose to obtain energy. Fermentation using Saccharomyces cerevisiae reach optimum result after 7 days. Rations digestibility increased with yeast addition 0.3% into fermentation substrate with moisture content 50% (Bidura et al., 2012; Martindah and Bahri, 2016).

Moisture content contribute significantly on growth of microbe in fermentation process. Metabolic reaction of microbial cell is assisted by water to transport nutrients into the cell as well as metabolic product transported out of cell. Each microbe has its own optimum moisture content to grow and regenerate. Low moisture content in the growth media hamper microorganism activity which in turn decrease the chance to degrade fiber and crude lipid components in the substrate. High moisture content on the other hand tend to decrease medium porosity causing difficulty in aeration process and mass transfer on microorganism metabolism (Vu et al., 2010).

2. EXPERIMENTAL SECTION

2.1 Materials preparation and formulating rations

Preparation of eggshell powder was initiated by washing dirt and removing membranes embedded. The eggshell was then soaked in hot water (100 °C) for 15-30 minutes, dried and grinded to obtain fine particles. Preparation of powder from leaf of lamtoro, kale and cassava were conducted by cutting the leaf from its stalk. The leaf was dried, grinded and sieved to

collect powder in fine texture. Similar procedure was carried out on other rough raw materials such as corn, coconut pulp, water hyacinth, palm kernel cake and golden snail.

Raw materials prepared in fine powder from the previous step were weighed in certain amount. The powders were mixed initially from smallest composition i.e. premix by eggshell powder and then water hyacinth powder followed by palm kernel cake powder, kale leaf powder, cassava leaf powder, coconut pulp powder, lamtoro leaf powder, golden snail powder and finally, corn powder. The portion of each powder was displayed on Table 1 while rations nutrients content was appeared on Table 2.

Table 1. Local raw material composition in rations formulation

Raw materials	Composition (%)
Fine corn	57
Coconut pulp powder	5
Palm kernel cake powder	4
Golden snail powder	17
Kale leaf powder	4
Water hyacinth powder	3
Cassava leaf powder	4
Lamtoro leaf powder	5
Eggshell powder	0.5
Premix	0.5
Total	100

Table 2. Nutrient composition of rations

Nutrients	Composition
Metabolic energy (EM) Kcal/kg	2921.39
Crude protein (%)	17.11
Crude fiber (%)	9.43
Crude lipid (%)	7.82
P available (%)	0.3
Ca (%)	0.73
Methionine (%)	0.21
Lysin (%)	0.53

Remark: Analysis result from laboratory of nutrition and animal feed University of Sriwijya 2016

2.2 Moisture content determination and the process of fermentation

Moisture content was determined by analyzing the rations dried matter value prior fermentation, so moisture can be calculated accordingly. Porcelain crucible was pre-heated to 105 °C oven for 1 hour and then placed in desiccator for 30 minutes. Porcelain crucible was weighted and mark as A. 5 grams samples (B) placed into the crucible and heated to 105 °C for 24 hours and then cooled in desiccator for 30 minutes. The final weight of porcelain crucible with sample were measured and mark as C. Moisture content was calculated according to following formula (AOAC, 1995):

$$Moisturecontent(\%) = \frac{B-(C-A)}{B} x100\%$$

$$Driedmatter(\%) = 100\% - Moisturecontent(\%)$$

Water to be added was calculated according to formula (Rostini et al., 2009):

$$\text{Rations moisture content (g)} = \frac{\text{BKrations(\%)}}{\text{BKrationsprepared(\%)}} \text{ x rations in total (g)}$$

Water to be added (mL) = rations moisture content (mL) – rations in total (mL)

Fermentation process was conducted according to previous report (Bidura et al., 2012) with modification i.e. rations was added by warm water (50–60 °C) based on previous researcher (Rostini et al., 2009), formula. Rations and water were stirred, and air cooled for 5 minutes. Yeast was added in form of fine particles 0.3% by weight of rations and then homogenized. The tray containing fermented rations with various moisture content was weighted and covered with aluminum foil and stored for 7 days (Kusumaningrum et al., 2012). After fermentation completed, the cover was removed, and the sample was dried in oven 45 °C for 6 hours. Rations was then subjected to analysis of crude protein, crude lipid and crude fiber.

2.3 Research method and data analysis

Research was planned by using completely randomized design with 5 treatments and 3 repetitions. The rations used in this research was marked as:

- P0 = Rations fermented without water addition (control)
- P1 = Rations fermented with moisture content 40%
- P2 = Rations fermented with moisture content 50%
- P3 = Rations fermented with moisture content 60%
- P4 = Rations fermented with moisture content 70%

Data was analyzed by using analysis of variance and if the treatment showed statistically significance difference, data was further analysis with Duncan's multiple range test.

2.4 Variable observed

The observed variable was dried matter, organic matter, crude protein, crude lipid, crude fiber, Nitrogen-free extract, microbe total, total acid and acidity (pH) (AOAC, 1995). Nutritional value was calculated according to following formula (Nelson and Suparjo, 2011): Nutrition value change (%) $= \frac{(AxBKoxCo)-(BxBKtxCt)}{(AxBKoxCo)} x10$

Note:

A = rations weight before fermentation (g)
B = rations weight after fermentation (g)
BK0 = dried matter before fermentation (%)
BKt = dried matter after fermentation (%)
C0 = nutrition value before fermentation (%)
Ct nutrition value before fermentation (%)

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3. RESULTS AND DISCUSSION

Average value of microbe total, total acid and pH analyzed from samples are display in Table 3. Rations samples were treated and fermented with different moisture content as described on procedure above.

Analysis of variance showed that rations fermented in different moisture content has significance difference (P<0,05) towards microbe total. Furthermore, analysis result exhibited that rations with 50% moisture content has higher microbe total compare to P0 and P1 but showed no statistically significance difference towards P3 and P4. 50% water in fermentation process presume to provide optimum condition for microbe growth. High level of moisture content moreover, hampered aeration and mass transfer on metabolism activity of corresponding microorganism, while low level of moisture content will make microorganism difficult to grow on the media (Vu et al., 2010).

Physical quality of rations fermented in 40% moisture content indicate slightly sour smell, light brown and dry texture. The rations fermented in 50% moisture content showed similar appearance both on smell and color but has wet texture. 60-70% moisture content fermentation resulted rations with strong sour smell, pale color and highly wet texture. The last two results indicate a decrease in nutrient quality along with growth of harmful microbe. Julendra et al. (2012) reported harmful microbe could cause damage on rations quality which can be seen from its color, smell change and decay process due to chemical composition modification. Furthermore, good quality rations physically can be assessed from sour smell, light brown color and wet texture (Saad et al., 2016).

The result indicated that microbe was grew in lag phase up until 40% moisture content. The number of microbe growth was not increased at this phase. Lag phase defines as slow growth of microbe due to adaptation and adjusting themselves to the environment. At 50-70% moisture content, the microbial growth is in stationary phase i.e. growing rate equals to mortality rate hence at this phase, the microbe growth is constant (Mathivanan et al., 2006).

Rations prepared from local raw materials with various moisture content according to analysis of variance is showed statistically significance difference towards total acid (P<0.05). Further test indicated that 50% moisture content treatment has distinctly higher total acid compare to P0 and P1 but not significantly different with P3 and P4. The increase of total acid was assumed due to high moisture content of the substrate which prompt the microbial growth and produced organic acids. Microbes used simple sugars in fermentation process to produce organics acids (Anggraeni and Yuwono, 2014).

Total acid titrated is total acids both dissociated and undissociated henceforth total acids bonds to NaOH can be identified. Our work obtained total acid at 1.47-2.70% higher than reported previously [4] whom obtained total acid of cacao beans pulp fermented with yeast at 0.23-0.62%. During fermentation process, organics acid is formed including lactic acid, acetic acid and butyric acid. These acids were produced due to Acetobacter activity in the yeast which has oxidative character (Feng et al., 2007).

Fermentation with 50% moisture content smelled sour which indicate high lactic acids was produced. 60-70% moisture content further, smelled very sour which indicate acetic acids and butyric acids were produced. Sour smell indicates the lactic acids formation while smelled very sour indicates the formation of acetic acids and butyric acids. Analysis of variance conclude, rations made from local raw materials fermented with various moisture contents showed significance difference towards pH (P<0.05). Further statistical analysis reveals fermentation with 50% moisture content (P2) significantly higher than P0 and P1 but not significantly different with P3 and P4. The rationale behind this fact is moisture can increase fermentation rate henceforth the higher moisture content, the more acids (low pH) rations obtained. The pH of fermentation is proportional to the total acid, which makes lower pH indicate total acids produced is higher. The decrease of pH started at 50% moisture content as the increase of total acids produced. Phenomenon as the decrease in pH is accompanied with the increase in total acid on sago fermentation. pH was decreased due to fermentation aerobically produced oxygen which formed organic acids compound and affected pH value (Chaing et al., 2010).

Acidity scale (pH) is one of important factor in fermentation process (Azizah et al., 2012). In this research we found out the average of rations pH fermented in different moisture content is 4.42-6.20. pH started to decrease at 50% moisture content through 70% i.e. 4.48-4.42. pH measured in cempedak skin fermentation to obtained bioethanol by using *Saccharomyces cerevisiae* is 4-5.

Nutritional changes of rations were analyzed before and after fermentation in different moisture content is displays in table 4.

Analysis of variance result concluded that rations fermented in different moisture content statistically significance difference (P<0.05) towards crude protein content. Moisture affects the increase and decrease of crude protein content resulted from microbial activity. Further test exposes rations P2 (50%) higher significantly (P<0.05) on its crude protein compare to others. P1 also showed significance difference (P<0.05) compare to other treatment while P0 reveals significance difference compare to other (P<0.05). P3 has no significance difference (P>0.05) compare to P4 in the decrease of crude protein content.

The highest change of crude protein content was discovered on P2 i.e. 5.65% of initial value (17.11% into 17.60%). The condition of P2 henceforth indicate the optimum condition among other treatments for Saccharomyces cerevisiae. Water obviously help microbial growth and metabolism in fermentation. The increase of crude protein strongly associated with microbial activity. Our analysis also found out that microbe total at P2 is 7.20×10^{10} CFU g^{-1} while control (P0) shows 3.23×10^{10} CFU g^{-1}. The increase in amount of microbe directly correlated to the increase of protein-rich microbial mass

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Table 3. Average value of microbe total, total acid and pH rations prepared from local raw materials with different moisture content on fermentation

Treatment	Variable		
	Microbe total (10^{10} CFU g^{-1})	Total acid (%)	pH
P0	$3\,23^{a}$±1.08	1.53^{a}±0.06	6.20^{b}±0.05
P1	3.20^{a}±0.09	1.47^{a}±0. 04	6.27^{b}±0.10
P2	7.20^{b}±1.08	2.42^{b}±1.02	4.48^{a}±0.02
P3	7.37^{b}±1.23	2.57^{b}±1.07	4.46^{a}±0.03
P4	7.43^{b}±1.11	2.70^{b}±1.18	4.42^{a}±0.04

Note: Different superscript in the same column indicate significance difference.

Table 4. Nutritional changes of rations fermented from local raw materials

Treatment	Change					
	Crude protein (%)	Crude lipid (%)	Crude fiber (%)	Dried matter (%)	Organic matter (%)	NFE (%)
P0	0.01^{c}±0 .90	0.02^{a} ±0.86	-0.01^{b}±0.94	0.01^{c}±0.03	0.01^{c}±0.03	1.87^{c}±0.12
P1	-2.93^{b}±0.50	2.5bc±0.94	3.95^{c}±0.27	-0.28^{b}±0.01	-0.42^{b}±0.04	-6.76^{b} ±0.22
P2	-5.65^{a}±0.20	4.55^{c}±0.66	5.68^{d}±0.56	-2.70^{a}±0.15	-3.72^{a}±0.13	-11.85^{a}±0.27
P3	1.54^{d}±1.19	2.06ab±1.98	-1.85^{a}±1.33	2.98^{d}±0.13	3.48d±0.07	13.66^{d}±0.06
P4	2.53^{d}±0.48	1.26ab±0.86	-3.06^{a} ±0.23	4.42^{e}±0.01	5.88^{e}±0.06	19.71^{e}±0.20

and so does crude protein content. Mass of cell composed of protein compound which makes large amount of microbe correspond to large amount of protein content (Wajizah et al., 2015). Fermentation process drives microbe to produced enzyme used to degrade complex compound into simpler one and to synthesis protein and protein enrichment. Protein enrichment was achieved from protein synthesis by microbe which produced degradation enzyme used on converting complex compound into smaller one (Heres et al., 2003).

Crude protein was decreased on P3 and P4. High content of moisture tends to accelerate not only the growth of useful microbes but also harmful microorganism, this situation caused microbial growth to increase microbial mass is hampered. High moisture content can reduced substrate porosity which in turn decrease oxygen exchange and increase risk of bacterial contamination (Juliarti and Alfaizah, 2013). Physical quality appearance of our product exhibited the possibility of nutrient quality reduction due to the existence of green and dark brown mycelia fungus, smells very sour, pale color and very wet texture on the fermented rations. Similar occurrence i.e. contamination of mold producing-toxin caused damage and made the rations quality decline physically, chemically and biologically and reduced palatability and the diminish of nutrient value (Martindah and Bahri, 2016).

Analysis of variance confirmed that moisture content treatment statistically significance difference ($P<0.05$) towards crude lipid content. This fact strengthens the notions that moisture content indeed affects the reduction of crude lipid caused by microbial activity. Statistical test further verified that the treatment of rations at P3 not significantly different ($P>0.05$) to P0 and P4 ($P>0.05$). P1 however is largely decrease crude lipid in significant way ($P<0.05$) compare to P3 and P4 but lower significantly compare to P2 ($P<0.05$). P2 has crude lipid much lower significantly ($P<0.05$) compare to P1.

The highest crude lipid change is obtained at P2 i.e. 4.55% of initial value (7.82% into 7.27%). P2 with 50% moisture content was able to provide optimum condition for microbe to hydrolysis complex crude lipid into smaller volatile fatty acids. The presence of water in fermentation process produced volatile acids i.e. sort chain fatty acids which easily to vaporize. Crude lipid content of rations consists of glycerol, fatty acids and vitamin which is soluble in volatile fatty acids. Water promote crude lipid hydrolysis into glycerol and free fatty acids (Rostini et al., 2009).

Moisture content treatment marks as P0, P1, P3 and P4 exhibited change of crude lipid content but not as high as P2. Moisture in less or more than 50% seems to hinder microbial optimum growth. Moisture plays important role in substrate decomposition process. Substrate generally contain organic substance which is used by microbes for its growth and regeneration. Saccharomyces cerevisiae utilize lipid in the substrate as energy resource on metabolism process in the cell. Low moisture content tends to hamper nutrient transport hence microbial growth is slower and cannot degrade crude lipid optimally. High moisture content further reduces decomposition rate of substrate and limited nutrient availability for microbe. Mass transfer in metabolic process also harder to carried out under high content of moisture.

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Treatment of moisture content confirms to give significance difference (P<0.05) towards crude fiber content change according to analysis of variance result. Statistics proved that moisture affects the increase and decrease of crude fiber content due to microbial activity. Further test result informed that the significance difference achieved highest value at P2 towards the decrease of crude fiber. It is also can be concluded that P1 showed significance difference (P<0.05) to other treatment as well as P0 to other treatments while P3 showed no significance difference (P>0.05) to P4 towards increment of crude fiber content.

The highest crude fiber content decline by treatment P2 i.e. 5.68% (9.34% into 8.66%) indicate microbial activity runs optimally under 50% moisture condition. Moisture takes important part in biosynthesis and enzyme secretion (Idiawati et al., 2014). Saccharomyces cerevisiae showed secondary activity i.e. produced cellulase which assists complex compound degradation into smaller compound (Nuryana et al., 2016). The mold also able to hydrolyze cellulose to form glucose and indirectly reduced crude fiber content. Cellulose compose of carbohydrate, useful substrate for microbial growth as feed source (D et al., 2015).

Treatment P3 and P4 exhibit increase of crude fiber content. Hight content of moisture appears to prevent optimum activity of microbe to degrade crude fiber. Total microbe in P4 was calculated as highest $7.43x10^{10}$ CFU g^{-1} . The microbe in this number allegedly is not the useful microbes which assisted fermentation. Physically appearance exhibit there are harmful fungal mycelia formed in the substrate. The growth of fungal mycelia contributes to crude fiber mass in the fermentation media (Samadi et al., 2015). Microorganism cell wall is fiber source which contribute fiber content on fermentation product (Rostini et al., 2009).

Analysis of variance result for moisture content effect on dried content change shows significance difference (P<0.05). Further test concluded the increase of dried content fermented in P2 treatment is higher (P<0.05) compare to P1. The decrease of dried matter found out on P4 with a larger decline compare to P3 and P0 (P<0.05). P2 increase the dried content by 2.70% from initial value before being fermented (90% into 95.02%) while P4 decrease the dried content by 4.42% from initial value before it fermented (90% into 87.73%).

Dried matter value raises when fermented in 50% moisture content. At this condition, oxygen diffusion presume to be optimal and provide a smooth process of heat exchange which cause moisture content decrease. During fermentation water used by microbes for metabolism activity. The activity prompt oxygen diffusion runs well and then decrease the content of moisture. Another author described that dried matter of substrate increase due to microbial activity in solid medium used water in the substrate and made it less water to contained (Saad et al., 2016).

Dried matter decreased in P0, P3 and P4 treatments. Fermentation in 60-70% moisture content caused rations dried matter to decrease. High content of moisture possibly hinders the oxygen diffusion which make heat transfer was disturbed and lower the activity and microbial growth. High moisture content can also hamper aeration process which affects evaporation and keep the rations moisture content stays high (Juliarti and Alfaizah, 2013). P0 was found out to have its dried matter decreased. Low content of moisture obviously inhibits microbial growth. The moisture plays important role on nutrient transport, chemical process and metabolic activity during fermentation (Idiawati et al., 2014). Condition in lack of water decreased microbial growth as well as substrate degradation. Throughout fermentation the component of rations was degraded by mold, typically carbohydrate to form glucose and by product such water which reduced dried matter content (Bidura et al., 2012).

The variance was analyzed statistically and reveals that moisture content give significance difference towards organic matter (P<0.05). Further statistical test concluded organic matter increase in P2 which is higher than P1 (P<0.05). P4 displays decrease on its organic matter more than P3 and P0 (P<0.05). Organic matter increases by 3.72% in P2 treatment compare to before fermented (94% into 94.9%) while organic matter decreases by 5.88% in P4 treatment compare to before fermented (94% into 92.55%)

Fermentation in 50% moisture content raise the organic matter indicate optimum microbial activity to produce enzyme which converts organic molecule into smaller compounds. The conversion of organic molecule produced simpler substances such as fiber, lipid and carbohydrate and increase organic matter of rations (Nuryana et al., 2016).

Moisture content of 60-70% in contrary decreases the organic matter. High level of moisture in fermentation tends to accelerate air exchange which promotes evaporation. Droplets were formed on the tray and drips onto substrate surface and contribute to moisture content (Feng et al., 2007). This condition is assisting the accretion of harmful bacteria and prevents the optimum microbial growth. The increase of protein-rich microbial mass is therefore decelerated and lower one of organic mass source in the rations. Physical appearance exhibits some fungus grew with sour's smell, pale color and wet texture in the fermented rations. It had been reported that bacterial contamination cause decay on rations quality including color aberration, change of smell, and decomposition due to chemical composition modification (Julendra et al., 2012). In addition, organic matter decrease throughout fermentation can be caused by harmful microbe proteolytic activity which grew during the process. Calculation of total microbes showed that at P4, it contains $7.43x10^{10}$ CFU g^{-1}. The change of dried matter and organic matter show similar tendency. The change of organic matter is aligned with the change of dried matter (Nelson and Suparjo, 2011).

Calculation by using analysis of variance informed that moisture content show significance difference towards Nitrogen-free extract (P<0.05). Further test confirm rations P2 has NFE highest increased (P<0.05) compare to P1 while P4 has NFE lowest decreased (P<0.05) compare to P3 and P0. The NFE

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increase by 11.85% from its initial value on P2 (50.36% into 54.85%) while NFE decrease by 19.71% from it initial value on P4 (50.36% into 42.30).

Microbial growth at P2 condition reach optimum due to good performance of its metabolic activity and enzymatic hydrolysis process (Kusumaningrum et al., 2012). The increase of NFE implied by the decrease of crude fiber on P2 by 5.68%. The decrease on crude fiber was triggered by cellulase enzyme produced by microbes and was able to break down cellulose into glucose which then use as carbon and energy resource (Sitohang et al., 2012).

The NFE decreased in 60-70% moisture content of fermented rations evidence that watery substrate reduces the microbial activity. Microbial activity to produce enzyme was hampered hence degradation of complex compounds cannot optimally conducted. Fiber degradation is one of process being hampered process which reduce the yield of NFE. The total microbe also is not correlates to moisture content treatment as in P4. The number of microbe was calculated as highest i.e. $7.43x10^{10}$ CFU g^{-1}. High number of microbes does not effective proportionally in degrading complex compounds (Julendra et al., 2012).

Generally, low NFE does not give advantage for the microbe because it is also means organic matter digestible component is low and reduce the amount of energy obtained. NFE is affected by moisture content, ash content, crude protein, crude lipid and crude fiber (Saad et al., 2016).

4. CONCLUSIONS

Fermentation of local raw materials rations in 50% moisture content can increase total microbes, total acidity, crude protein, dried matter, organic matter and Nitrogen-free extract. The process in contrary decreases crude lipid, crude fiber and pH.

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