The effect of aerated storage system and turmeric (Curcuma longa L.) addition on the quality of lactic acid bacteria fermented feed

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Abstract. This study determined the effect of turmeric (Curcuma longa L.) in preventing the rancidity process of concentrate feed based on lactic acids bacteria fermentation (CFLAB) in aerated aerobic storage conditions. The treatments were the addition of pure turmeric, extract turmeric, and vitamin E in feed with the level of 0, 0.5, 1, and 2%, with three replications. Aeration was done every morning and evening for 40 days. Rancidity observation was carried out after 40 days. The parameters observed were pH values, numbers of free fatty acids (FFA), and physical quality. The addition of antioxidant sources during aerated aerobic storage affected the FFA content. The addition of three sources and five levels of antioxidant sources maintain a pH value in the neutral range during storage. Based on the FFA analysis, the addition of turmeric and turmeric extract was suppressed increasing of FFA value better than control and vitamin E. This research concludes that the provision of antioxidant sources inhibit the increasing levels of FFA during storage. The results of the organoleptic test showed that the quality of CFLAB persisted quite well in all treatments with aerated aerobic on 40 days storage.

1. Introduction

Concentrate feed based on lactic acid bacteria fermentation (CFLAB) is one of the ruminant rations formulated with rice bran as the most ingredient and some fat-source as raw materials. Rice bran was a by-product of the rice milling process, so its availability fluctuates throughout the year according to the harvest season. When the harvest season is coming, rice bran is abundant and the price is relatively low, but while in the dry season, the amount of this feedstuff decreases and the price is high.

Besides that problem, another problem found in the rice bran is instability of the quality during the storage process. Instability is caused by the activity of enzymes that can damage its quality, such as the presence of an oxidative process of rancidity. This instability was mainly due to the presence of lipase enzymes found in rice bran and also peroxidase enzyme, which can cause oxidative damage and rancidity in the feed. The used of synthetic antioxidants is widely used in feed additive, but its use was limited because several synthetic antioxidants can cause liver damage and carcinogenic if given in high amounts [1]. Thus, it is necessary to provide natural antioxidants that do not harm the body of the animal.

Turmeric (Curcuma longa L.) is a plant that belongs to the genus Curcuma with the family Zingiberaceae. Although the bioactive compounds in turmeric has not been widely studied, existing
studies have found that essential compounds are curcumin and essential oils [2]. In general, the essential oils found in turmeric are mixed complexes of a monoterpene, sesquiterpene, and odortic compounds, which function as strong antioxidants, anti-inflammatory and anti-bacterial activities [3]. The use of turmeric in the ration as a source of antioxidants is expected to reduce the activity of rancidity in the ration to increase the resistance of the ration in more extended storage with aeration treatment in aerobic conditions. The objective of the study was determined the effect of additional of turmeric in preventing the rancidity process of CFLAB in aerated aerobic storage conditions.

2. Material and methods

2.1. Material

Turmeric powder is prepared by grinding the dry turmeric bought from the local market. Extract turmeric powder is prepared with the extraction method. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and vitamin E (alpha-tocopherol 95%, Sigma Aldrich, Singapore) were used for preparation.

2.2. Methods

The sample is a mixture of CFLAB (100 g) which was made in open conditions with aerobic aeration for 40 days. The treatments were the addition of a source of antioxidants (turmeric powder, extract turmeric powder, and vitamin E) with different concentrations (0.0, 0.5, 1.0, 1.5 and 2.0%) into the wet ration. The total number of treatments was 15 with three replications each. Samples analyses were carried out regularly in a ten-day lag time. Tests were carried out in the form of organoleptic, acidity (pH), and free fatty acid (FFA). Table 1 shows the antioxidant sources and levels given to CFLAB. Each level of antioxidant were storage at five different periods, and each treatment has three replications. The aeration treatment accomplished by flipping the sample back every morning and evening for 40 days.

| Table 1. Level of the addition of antioxidant sources to concentrate feed based on lactic acids bacteria fermentation (CFLAB) |
|-------------------------------------|
| Antioxidant sources | Antioxidant Level addition (%) |
| Turmeric powder | I  | II  | III | IV | V |
| Extract turmeric powder | 0  | 0.5 | 1   | 1.5 | 2 |
| Vitamin E | 0  | 0.5 | 1   | 1.5 | 2 |

Organoleptic parameters that were observed included color, odor, texture, and fungus. Five organoleptic panelists assessed the CFLAB based on the questionnaire (Table 2). The organoleptic parameters were tested on the 0, 10, 20, 30, and 40 days of storage. The pH value was tested by weighing 1 g sample and added the aquades in each sample inside the tube. The sample was mixed using a vortex until homogen. The pH value was tested using a pH meter. The FFA value was tested by weighing 1 g sample and put into Erlenmeyer, then added the hot, neutral alcohol 25 ml and phenol-petaelin indicator 1 ml. The sample was mixed using a vortex and then titrated using 0.05 N NaOH until the pink color was obtained.

| Table 2. Organoleptic score assessment questionnaire for concentrate feed based on lactic acids bacteria fermentation (CFLAB) analysis |
|-------------------------------------|
| Score | Color | Odor | Texture | Fungus |
| 1 | Black | Rancid | Slimy and clotted | So many |
| 2 | Blackish brown | Neutral | Clotted | Many |
| 3 | Faded brown | A little sour | A little clotted | A lot |
| 4 | Brown | Sour | Mesh, a little clotted | A little |
| 5 | Yellowish-brown | Fresh sour | Mesh, not clotted | Nothing |
2.3. Statistical analysis
The data were submitted to analyses of variance of factorial pattern followed by Duncan’s multiple range test (DMRT) post hoc tests considering P < 0.05.

3. Results and discussion

3.1. Organoleptic test
The color was classified in good condition during storage. The color change was better in turmeric powder and extract turmeric powder than control. Utomo et al. [4] reported that silage color was good when the color of silage product was the same with the beginning silage. Despal et al. [5] added that it the darker color produced indicated the low quality of silage. The color quality of CFLAB during storage has good quality; the higher level of turmeric given has good results to maintain the color of CFLAB during storage compared to vitamin E.

The more antioxidant added, the better quality of the odor produced. During storage, the quality of the odor produce was decreased. Tuorilla and Cardello [6] cit. Haman et al. [7] reported that rancidity is a reaction that occurs because of the presence of oxygen in the air which reacts with the fat, and produces an odor at a certain level so that consumers cannot accept it. The differences in odor was influenced by the antioxidants in turmeric. Cikrikci et al. [8] reported that the antioxidant levels in turmeric were 3 to 5%. Jayaprakasha et al. [9] reported that antioxidant activity in turmeric prevents excessive peroxidation of fatty acids, thus, the rate of rancidity can be reduced.

One of the indicators to determine the quality of CFLAB during storage was texture. All treatments showed good texture. With the absence of clots at the beginning of storage until the 10th day. Decreasing the quality of the texture begins on the 20th day with a few lumps until the end of storage with more clots. The addition of antioxidant sources in CFLAB could maintain the CFLAB texture until the 10th day in aerobic aeration storage. Solihin et al. [10] stated that texture determines the physical quality of feed. Dense texture is possible to be more durable in the process of handling, storage, and transportation. The feed was damaged if it showed a deviation that crosses the line, such as the number of clots caused by moisture based on the observation of texture.

The addition of types and levels of antioxidant sources had an adverse effect to the physical quality and was unable to maintain fungal growth in CFLAB until the 10th day of aerobic aeration storage. It can be caused by direct contact between the sample and the environment and makes the fungi easy to grow. Storage of the 20th to 40th day increased the number fungi, which more fungi were observed at the end of storage. Trisyulianti et al. [11] reported that the fungi grow faster in food with high water content, which was around 32.5%. Feed storage decreased the quality of feed, as indicated by the presence of microbial contamination in the feed. Solihin et al. [12] stated that during the storage, animal feed changed due to microbial activity. Handayani et al. [13] stated that the contamination of toxin-producing fungi caused the feed to be inappropriate for livestock due to modification of chemical composition. The antibacterial activity of curcumin (diferuloylmethane) plays a role in inhibiting the stability and assembly of FtsZ protofilaments as essential factors in bacterial cell division [14]. Vitamin E has no antimicrobial activities such as antifungal and antiviral. Radwan et al. [15] stated that turmeric and its extract showed the largest zone of inhibition of fungal growth. Based on the organoleptic test, the addition of antioxidant sources in CFLAB has a reasonably good quality. Turmeric powder and extract turmeric show better organoleptic results than vitamin E. The higher the level of the source of antioxidants given, the better in maintaining organoleptic results. It happened to all sources of antioxidants.

3.2. The pH value
The pH value 0th day indicated that extract turmeric powder, turmeric powder, and vitamin E had an average of 4.84 ± 0.21 due to the accumulation of fermentation product from CLAB (Table 3). The pH value on the 10th day was higher than the 0th day, but it was not different with the 20th day to the 40th day. This finding indicated that the storage time did not affect the pH value. On the 10th day, the mean
pH was 8.02 ± 0.23, and the value was in the range above neutral pH. The storage time maintained pH from the 10th day to the end of storage in the neutral pH range.

The antioxidant levels and sources did not affect the pH value. It is getting increased storage time will significantly increase the pH value at the 10th day storage, but at 40th day of storage the increase in pH did not show significant differences. Weinberg and Muck [16] reported that when silage was exposed to oxygen, the aerobic organisms grew by utilizing the existing substrate and causing the pH increase. These microorganisms include yeast, mold, and various aerobic bacteria (Bacilli, acetic acid bacteria, and listeria) that develop by utilizing sugar in plants, fermented products, and other compounds produced during storage. Ni et al. [17] stated that ripening increased the number of molds and increase the pH. Molds caused an aerobic atmosphere and reduced the silage nutritional value [18]. This shows that CFLAB with an antioxidant source is not resistant to aerobic conditions and needs to be done with anaerobic conditions.

Table 3. The effect of different antioxidant sources, different level of antioxidant addition and different storage periods on pH value of concentrate feed based on lactic acids bacteria fermentation (CFLAB)

| Antioxidant Sources | Value | Storage periods (day) | pH | Average |
|---------------------|-------|-----------------------|-----|---------|
|                     | 0     | 10                    | 20  | 30      | 40 |
| Extract             | 0.49 ± 0.18 | 8.32 ± 0.14 | 8.51 ± 0.20 | 8.54 ± 0.44 | 8.61 ± 0.20 | 7.78 ± 1.53 |
| Turmeric powder     | 0.49 ± 0.15 | 8.10 ± 0.27 | 8.43 ± 0.33 | 8.43 ± 0.16 | 8.46 ± 0.43 | 7.67 ± 1.47 |
| Powder              | 1.87 ± 0.33 | 7.98 ± 0.27 | 8.23 ± 0.45 | 8.38 ± 0.39 | 8.40 ± 1.86 | 7.27 ± 1.53 |
| Powder              | 1.87 ± 0.35 | 8.07 ± 0.22 | 8.43 ± 0.14 | 8.54 ± 0.31 | 8.55 ± 0.50 | 7.69 ± 1.52 |
| Powder              | 2.89 ± 0.55 | 8.16 ± 0.41 | 8.45 ± 0.13 | 8.48 ± 0.67 | 8.56 ± 0.29 | 7.61 ± 1.73 |
| Average             | 4.79 ± 0.32 | 8.13 ± 0.24 | 8.42 ± 0.23 | 8.48 ± 0.33 | 8.22 ± 0.97 | 7.60 ± 1.49 |

Average: 4.88 ± 0.17 | 8.02 ± 0.22 | 8.26 ± 0.21 | 8.33 ± 0.31 | 8.33 ± 0.17 | 7.56 ± 1.37

Average total: 4.84 ± 0.21 | 8.02 ± 0.23 | 8.38 ± 0.21 | 8.43 ± 0.28 | 8.25 ± 0.91 | 7.58 ± 0.25

3.3. Free fatty acid (FFA)
The 0th day storage time showed significantly different in FFA levels with a mean value of 2.72 ± 0.91 compared with CFLAB levels on the 5th and 40th days (Table 4). It occurred because of the production of lactic acid during the fermentation process. FFA production that occurs on day 0th is a product of CFLAB fermentation. Aeration treatment decreased the lactate products, and the fat contained in CFLAB can be damaged or depleted. Addition of antioxidant sharply reduced FFA levels on day 5 with a mean of 0.52 ± 0.24 and 40th day with a mean of 0.57 ± 0.32. The CFLAB storage reduced FFA levels on the 5th and 40th day and showed a significant difference with the control (P < 0.05).

The FFA value decreases in all samples at each addition of antioxidant source levels and storage time in aerobic aeration conditions. This was caused by the presence of organic compound activity. When compared with controls, the addition of turmeric powder and extract turmeric powder was effective in inhibiting the rancidity process due to the inhibition of enzymatic activity. Kim et al. [19] reported that the difference in lower FFA levels compared to controls showed that the treatment was effective in slowing enzymatic degradation.

The different environmental influences creates the fluctuations of FFA value in the storage period. Decreased in FFA value can be caused by the influence of antioxidants added to the sample and can also be caused by the influence of aerobic storage. Frankel [20] cit. Haman et al. [21] stated that when
free radicals are produced, the presence of oxygen caused the formation of peroxide or hydroperoxide. These compounds are unstable and easily broken down to produce more free radicals, commonly referred to as "radical chain reactions." It showed that the addition of an antioxidant source in CFLAB can inhibit the rancidity process.

**Table 4.** The effect of different antioxidant sources, different level of antioxidant addition and different storage periods on FFA value of concentrate feed based on lactic acids bacteria fermentation (CFLAB)

| Antioxidant Sources | Level (%) | Storage periods | FFA (%) | Average |
|---------------------|-----------|----------------|---------|---------|
|                     |           | 0              | 5       | 40      |         |
| Extract urmeric powder | 0.5       | 3.47±0.13a   | 0.78±0.12b | 0.99±0.16c | 1.75±1.34 |
|                     | 1         | 3.27±0.15b   | 0.35±0.03a | 0.28±0.01a  | 1.30±1.52 |
|                     | 1.5       | 3.36±0.33b   | 0.35±0.11a | 0.28±0.01a  | 1.33±1.52 |
|                     | 2         | 2.90±0.07a   | 0.28±0.03a | 0.29±0.02a  | 1.15±1.35 |
| Average             |           | 3.29±0.26a   | 0.44±0.49a | 0.45±0.29a  | 1.39±1.38 |
| Turmeric powder      | 0.5       | 1.73±0.05a   | 0.28±0.02a | 0.29±0.01a  | 0.75±0.72 |
|                     | 1         | 1.69±0.08a   | 0.28±0.01a | 0.29±0.01a  | 0.75±0.72 |
|                     | 1.5       | 1.67±0.01a   | 0.29±0.01a | 0.30±0.02a  | 0.75±0.72 |
|                     | 2         | 1.63±0.08a   | 0.28±0.01a | 0.29±0.03a  | 0.73±0.70 |
| Average             |           | 2.04±0.76a   | 0.38±0.21a | 0.43±0.13a  | 0.95±0.91 |
| Vitamin E           | 0.5       | 3.47±0.10a   | 0.78±0.12a | 0.99±0.16a  | 1.75±1.34 |
|                     | 1         | 1.97±0.17a   | 0.70±0.01a | 0.78±0.01a  | 1.16±0.64 |
|                     | 1.5       | 3.82±0.02a   | 0.77±0.11b | 0.85±0.05b  | 1.81±1.56 |
|                     | 2         | 1.27±0.05a   | 0.84±0.02a | 0.99±0.10a  | 1.04±0.20 |
| Average             |           | 2.83±1.37a   | 0.76±0.06a | 0.88±0.11a  | 1.40±1.19 |
| Average total       |           | 2.72±0.91a   | 0.52±0.24a | 0.57±0.32a  | 1.28±1.17 |

Different superscripts (a-j) in the same column and row represent significant difference at P<0.05.

4. Conclusion
The concentrate CFLAB with the addition of turmeric powder and turmeric extract showed better results than the addition of vitamin E and control. Based on color, odor, texture, and fungus, it revealed that the quality of CFLAB persisted quite well in all treatments with aerobic aeration treatment at 40 days of storage. Based on pH value, the addition of the levels of antioxidant sources did not affect the but could maintain a pH value above the neutral pH range, and the storage time did not influence the pH. Based on the number of free fatty acid levels, the storage time has a significant effect on suppressing FFA value, more levels of antioxidant sources can reduce the number of free fatty acids compared to controls.

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References
[1] Krishnainah D, Sarbatly R dan Nithyanandam 2010 *J. Food Bioprod Proc.* In Press.
[2] Ma J, Wang Y, Liu Y, Gao S, Ding L, Zhao F and Qiu F. 2015 *J. Fitoterapia.* 103 90–96
[3] Angel G R, Menon N, Vimala B and Nambisan B 2014 *Ind. Crops Prod.* 60 233–238
[4] Utomo R, Budhi S P S and Astutji I F 2013 *J. Anim.* 37 173-180
[5] Despal I G, Permana, Safarina S N dan Tatra A J 2011 *Media Peternakan.* 43 69-76
[6] Tuorilla H and Cardello A 2002 *Food Quality Preference.* 13 561-569
[7] Haman N, Romano A, Asaduzzaman M, Ferrentino G, Biasiol F, Scampicchio M 2017 *Talanta Journal* 16 407-412
[8] Cikrikci S, Mozioglu E and Yilmaz H 2008 Record of Natural Products 2 19-24
[9] Jayaprakasha G K, Rao L J and Sakariah K K 2005 Trends Food Sci. Tech. 16 533–548
[10] Solihin, Muhtarudin and Sutrisna R 2015 Jurnal Ilmiah Peternakan Terpadu 3 48–54
[11] Trisyulianti, Suryahadi E B W H E and Rakhma V N 2003 Media Peternakan 26 35–40
[12] Solihin, Muhtarudin and Sutrisna R 2015 Jurnal Ilmiah Peternakan Terpadu 3 48–54
[13] Handayani S and Joko S 2000 Analysis keragaman kapang pencemar pakan unggas (Indonesia: Puslitbang Biologi LIPI)
[14] Rai D, Singh J K, Roy N and Panda D 2008 J. Bio Chem. 410 147–155
[15] Radwan M M, Tabanca N, Wedge D E, Tarawneh A H and Cutler S J 2014 Fitoterapia 99 341–346
[16] Weinberg Z G and Muck R E 1996 FEMS Microbiological Reviews 19 53–68
[17] Ni K, Wang F, Zhu B, Yang J, Zhou G, Pan Y, Tao Y and Zhong J 2017 Bioresource Technology 238 706–715
[18] Canibe N, Kristensen N B, Jensen B B and Vils E 2014 J. App Micro. 4 747–760
[19] Kim S M, Hyun J C and Seung T L Journal of Cereal Science 60 243–248
[20] Frankel E N Lipid Oxidation (California: Woodhead publishing) pp 15–7
[21] Haman N, Romano A, Asaduzzaman M, Ferrentino G, Biasioli F and Scampicchio M 2017 Talanta Journal 16 407–412