Evaluation of palm oil and water temperature in bioactive edible film preparation of total mixed ration pellet on hardness and microbial profile

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Abstract. The effects of palm oil and water temperature on preparation of bioactive edible film (BEF) in total mixed ration (TMR) pellets were investigated, with highlight on hardness and microbial composition. The research followed a completely randomized design (CRD) with factorial of 4 x 4 with 3 replications. The first factor was crude palm oil (M) with levels of 0%, 1.5%, 3%, 4.5%, while the second factor was water temperature (T) as a solvent for tapioca flour, namely 30 °C, 35°C, 40°C, and 45°C. The results showed that palm oil concentration and water temperature had a significant effect (P <0.05) and showed an interaction between the two factors on the hardness of BEF TMR pellets. Lactic acid bacteria (LAB) and fungi population decreased with the addition of palm oil content and increasing of water temperature. Coliform was not detected in all palm oil and temperature treatments. It could be concluded there was an interaction between addition of palm oil and water temperature to the hardness of BEF TMR pellets, with the best combination produced by 3% palm oil and 35°C water temperature. The population profile of LAB and fungi at treatment of 3% palm oil and 30°C produced the most satisfied pellet which also meets Indonesia National Standard (SNI) for ruminant feed.

1. Introduction

Complete diet feeding system for ruminants has received importance as it enables to stabilize rumen fermentation, diminish nutrition loss by fermentation, improve ammonia utilization, and reduce feed selection [1]. High water content of complete feed could lead to physical, chemical and also biological damage during storage and usage. The damage emerges due to the feed form, high environment humidity, and exposure to water and saliva. Complete feed storage in high humidity and temperature would promote growth of fungi enabling to produce toxic substrates [2]. Additionally, such condition accelerates formation of rancidity, decreases palatability and intake, reduces shelf life and ultimately disturb the animal health. To solve these challenges, coating the pellet by edible film can be a meaningful attempt since it allows to protect feed against microbial contamination and other potential damages. The use of pellet as complete diet has been reported to bring advantages, such as higher milk production, better economic benefits for farmers, reduced environment pollution and labor hours, as well as easier handling for transportation [3]. This study was to develop the preparation of complete feed using bioactive edible film (BEF) enriched with lactic acid bacteria (LAB) as a probiotic and carotenoid to produce better quality feed. Bioactive film is expected not only longer the feed shelf life and reduce the damage of perishable feedstuff but also provide bacteria that enhance digestive process [4]. Carotene as antioxidant was administrated to extend feed storage; besides, carotene in feed could play role as anti rancidity [5]. Choo (2000) stated that crude palm oil (CPO) contained considerable amount of carotene, up to 500–1600 ppm [6]. In previous study, LAB as a TMR protectant was implemented [7]. The result revealed that local LAB inoculants showed noticeable activities on maintaining stability of nutritional compositions in tropical conditions, as also demonstrated by commercially available L. plantarum.
FCC123. However, further studies are envisaged, enabling to improve TMR protection technique in tropic. In this study, physical and microbial profile would be determined in order to understand the effects of CPO and water temperature in bioactive edible film TMR preparation.

1.1. Problem of Research
1. Does the use of palm oil affect the hardness and microbial profile of the pellet?
2. Does the use of water temperature as BEF solvent affect the hardness and microbial profile of BEF pellet?
3. Do two factors studied, e.g. palm oil and water temperature, provide any interaction effects? And which treatment result in the most satisfying hardness and microbial profile in BEF pellet?

1.2. Purpose of Research
1. Evaluating the effect of using palm oil on the hardness and microbes profile of BEF pellet.
2. Evaluating the effect of using BEF solvent temperature on the hardness and microbial profile of BEF pellet.
3. Evaluating the interaction between palm oil and BEF solvent temperature on the hardness and microbial profile of BEF pellet.

2. Method

2.1. Preparation of BEF Pellet TMR
Total mixed ration (TMR) was set at 18.5% CP and 12.5% CF) for preparing pellet feed. CPO was added at following levels: 0%, 1.5%, 3%, 4.5% of TMR dry matter. The concentration of CPO followed previous study using the oil up to 5% [8]. The tapioca flour (7%) was dissolved with 5% (v/w) of Lactobacillus plantarum broth culture at density of 10^11 [9] at 30°C, 35°C, 40°C, or 45°C. As prescribed in former study [10], water temperature should be controlled at 30°C – 60°C to maintain feed quality parameters, including texture, palatability and content of anti nutrition. All ingredients were mixed with universal mixer (Bosch MUM6N11) for 10 min, and pelleted by pellet mill machine (Welljoin 120). Pellet was then packed by polyethylene plastic bag according to the treatment to be stored for 8 weeks in room temperature (26°C) for observation. Dry matter and crumblesness were tested on the 8th week, while microbial count (LAB, coliform and fungi population) was observed weekly.

2.2. Pellet hardness
Pellet hardness corresponded to amount of force required to break the pellet, tested using manual hardness tester. Sample was placed on millimeter sheet on flat surface, then 500 g-load scales was dropped from 20 cm height [11]. Weight and height of load scales were counted by formula:

\[ P = \frac{F}{A}; h = \frac{1}{2}gt^2 \]

Notes:
P : Pressure (Pa)  h : height (m)
F : Force (N)  g : gravitation (m/s^2)
A : Area width (m^2)  t : time (s)

Pellet hardnesses were determined by how many millimeter blocks sheet filled by pellet crumbs.

2.3. Microbial Analysis [12].
Samples (10 g) were blended with 90 ml of sterilized water and serially diluted in 10^1 to 10^9. The total lactic acid bacillus (LAB) was quantified using plate count on lactobacilli deMan Rogosa Sharp agar (MRS; Difco) incubated at 30 °C for 2 days under anaerobic cultivation (Anaerobox Jar.). The quantification of coliform followed method of blue light broth agar (BLB; Nissui Ltd.) incubated at
30°C for 24 hour, while mold population was counted on potato dextrose agar (PDA; Difco) and incubated at 30°C for 24 hours. Mold was distinguished from yeast by appearance of morphological colony and cell forming under microscopic observation. Colonies were counted as viable numbers of microorganisms in colony-forming unit per gram of fresh matter (FM).

3. Results and discussion

3.1. Pellet hardness
Pellet hardness serves as a key physical indicator, ensuring that it is suitable for target animals. The hardness relies on water content and feedstuff used. High index of water absorption reduces pellet hardness and produces soft pellet [13]. Feedstuff with small particles produces hard pellet, due to stronger particle bound during high pressure pelleting process [14]. The hardness of samples was presented in table 1.

| Water Temperature | M0  | M1  | M2  | M3  |
|-------------------|-----|-----|-----|-----|
| T0                | 66.91g | 63.91sf | 55.56de | 50.78ed |
| T1                | 68.75g | 63.91sf | 57.89de | 50.38ed |
| T2                | 71.78gh | 65.08gh | 58.13de | 47.06e  |
| T3                | 75.00gh | 66.67gh | 60.53g  | 43.33a  |

Notes: T0: 30°C; T1: 35°C; T2: 40°C; T3: 45°C; M0: CPO 0%; M1: CPO 1.5%; M2: CPO 3%; M3: CPO 4.5%.

As the results, interaction between CPO and water temperatures towards hardness occurred clearly. Increase in oil content was responsible for adverse impacts on pellet hardness, suggesting that higher level of CPO led to lower pellet hardnes. Conversely, higher water temperature would produce higher pellet hardness. Pellet prepared in absence of CPO at water temperature of 45°C resulted in the highest hardness. Oil is often added to enhance energy content. The ingredient is essential for ruminant nutrition in order to improve animal’s health and productivity, while also reducing enteric methanogenesis [15].

3.2. Lactid Acid Bacteria population
Lactid acid bacteria (LAB) provide pivotal role in maintaining the ecosystem balance in digestive tract. In general, they are characterized as follows: Gram positive, negative catalase, non-spore forming bacteria, no cytochrome, aerotolerant, anaerobic, microaerophilic; the bacteria also require complex nutrients like amino acids, vitamin (B1, B6, B12, and biotin), purin and pyrimidine [16]. Profile of LAB population during 8 weeks of experiment was presented on table 2. Based on table 2, LAB was sensitive to temperature, in which the increase in water temperature caused the decrease in LAB population. LAB could well grow if temperature requirement was fulfilled, with optimal range from 37°C to 42°C [9, 16]. The highest number of LAB was found in 30°C (T0). Storage for 8 weeks in room temperature tends to increase LAB, with highest population in T0M1, reaching up to 2.95 × 10^8cfu/g. Water temperature at > 40°C caused disappearance of LAB even since the first week.

3.3. Coliform Population
Coliform includes all bacteria in bacil form, and often characterized as Gram negative, non-spore forming, and able to ferment lactose into gas and acid in less than 48 h at 37°C. In this study, coliform activity was not detected (nd), in all treatments and 8 weeks of experiment as shon in table 3. Pelleting and combination between CPO and water temperature with LAB presence apparently could depress the harmful bacteria coliform. In this case, quality of the prepared pellet fitted the standard, being safe and feasible as alternative feed [17].
### Table 2. Lactic acid bacteria (LAB) population (cfu/g) in pellet feed.

| Treatments | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     |
|------------|-------|-------|-------|-------|-------|-------|-------|-------|
| T0M0       | nd    | nd    | 2.20 x 10^2 | 2.25 x 10^4 | 1.42 x 10^4 | 2.68 x 10^5 | 1.44 x 10^6 | 2.22 x 10^7 |
| T0M1       | nd    | nd    | 1.20 x 10^2 | 8.30 x 10^4 | 1.32 x 10^4 | 5.96 x 10^5 | 8.20 x 10^6 | 2.95 x 10^7 |
| T0M2       | nd    | nd    | 1.00 x 10^2 | 1.10 x 10^4 | 9.70 x 10^4 | 5.28 x 10^5 | 7.60 x 10^6 | 2.79 x 10^7 |
| T0M3       | nd    | nd    | 4.20 x 10^2 | 2.60 x 10^4 | 1.01 x 10^4 | 4.98 x 10^5 | 9.10 x 10^6 | 2.14 x 10^7 |
| T1M0       | nd    | nd    | 1.01 x 10^2 | 3.60 x 10^4 | 1.06 x 10^4 | 2.66 x 10^5 | 4.90 x 10^6 | 2.46 x 10^7 |
| T1M1       | nd    | nd    | nd           | 3.30 x 10^4 | 1.05 x 10^4 | 2.76 x 10^5 | 7.60 x 10^6 | 1.99 x 10^7 |
| T1M2       | nd    | nd    | nd           | 4.50 x 10^4 | 1.05 x 10^4 | 2.28 x 10^5 | 9.20 x 10^6 | 2.51 x 10^7 |
| T1M3       | nd    | nd    | nd           | 1.01 x 10^4 | 2.45 x 10^5 | 6.50 x 10^6 | 2.56 x 10^6 | 2.25 x 10^6 |
| T2M0       | nd    | nd    | nd           | Nd           | Nd         | 1.28 x 10^5 | 8.70 x 10^5 | 2.25 x 10^6 |
| T2M1       | nd    | nd    | nd           | Nd           | Nd         | 1.06 x 10^5 | 2.09 x 10^6 | nd         |
| T2M2       | nd    | nd    | nd           | Nd           | Nd         | nd         | nd         | nd         |
| T2M3       | nd    | nd    | nd           | Nd           | Nd         | nd         | nd         | nd         |
| T3M0       | nd    | nd    | nd           | Nd           | Nd         | Nd         | Nd         | nd         |
| T3M1       | nd    | nd    | nd           | Nd           | Nd         | Nd         | Nd         | nd         |
| T3M2       | nd    | nd    | nd           | Nd           | Nd         | Nd         | Nd         | nd         |
| T3M3       | nd    | nd    | nd           | Nd           | Nd         | Nd         | Nd         | nd         |

Notes: T0: 30°C; T1: 35°C; T2: 40°C; T3: 45°C; M0:CPO 0%; M1:CPO 1.5%; M2:CPO 3%; M3: CPO 4.5%.

Based on Indonesia National Standard (SNI 2014) [18], coliform at level of 10^5 cfu/g feed can be harmful to animals. In previous standard (SNI 2008) [19], coliform acts as indicator for excret, water, and feed pollutant. Lower coliform number showed better water and feed quality. Previous work [20] concluded that 6-7% feedstuff was at risk of coliform, which may cause acute diarrhea in animal. With oxygen presence, coliform actively grow in 37°C.

### Table 3. Coliform population in pellet feed (cfu/g).

| Treatments | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     |
|------------|-------|-------|-------|-------|-------|-------|-------|-------|
| T0M0       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T0M1       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T0M2       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T0M3       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T1M0       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T1M1       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T1M2       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T1M3       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T2M0       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T2M1       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T2M2       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T2M3       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T3M0       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T3M1       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T3M2       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |
| T3M3       | nd    | nd    | nd    | nd    | nd    | nd    | nd    | nd    |

Notes: T0: 30°C; T1: 35°C; T2: 40°C; T3: 45°C; M0:CPO 0%; M1:CPO 1.5%; M2:CPO 3%; M3: CPO 4.5%. nd: not detected.
3.4. Fungi population

Since the first week, pellet without CPO showed a huge number (too numerous too count/tntc) of fungi. Fungi growth was affected by temperature, humidity, and inhibitors [21]. Longer storage period leads to increase in water content, thereby accelerating microbial proliferation; thus, such condition accelerated feed damage [22]. Fungi in pellet with 1.5% CPO started to grow in second week and increased steadily then until they reached 10^7 on the last week of observation. Fungi population in feed with 3% and 4.5% CPO was not detected for the first five weeks and consistently showed a lower fungi population, less than SNI standard of 10^6 CFU/g. Oil palm contains vitamin E, carotenoid, and antioxidant, as well as unsaturated fatty acids which retarded fungi growth and rancidity of pellet.

Table 4. Fungi population in pellet feed (cfu/g).

| Treatments  | Weeks | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
|-------------|-------|----|----|----|----|----|----|----|----|
| M0T0        |       |    |    |    |    |    |    |    |    |
| M0T1        |       |    |    |    |    |    |    |    |    |
| M0T2        |       |    |    |    |    |    |    |    |    |
| M0T3        |       |    |    |    |    |    |    |    |    |
| M1T0        |       |    |    |    |    |    |    |    |    |
| M1T1        |       |    |    |    |    |    |    |    |    |
| M1T2        |       |    |    |    |    |    |    |    |    |
| M1T3        |       |    |    |    |    |    |    |    |    |
| M2T0        |       |    |    |    |    |    |    |    |    |
| M2T1        |       |    |    |    |    |    |    |    |    |
| M2T2        |       |    |    |    |    |    |    |    |    |
| M2T3        |       |    |    |    |    |    |    |    |    |
| M3T0        |       |    |    |    |    |    |    |    |    |
| M3T1        |       |    |    |    |    |    |    |    |    |
| M3T2        |       |    |    |    |    |    |    |    |    |
| M3T3        |       |    |    |    |    |    |    |    |    |

Notes: T0: 30°C; T1: 35°C; T2: 40°C; T3: 45°C; M0: CPO 0%; M1: CPO 1.5%; M2: CPO 3%; M3: CPO 4.5%. tntc: too numberours too count.

4. Conclusions

1. CPO addition considerably affected the hardness of BEF pellet, while also decreased fungi population. The best concentration was found to be 3%.
2. Water temperature altered the hardness of BEF pellet feed. Increasing water temperature caused decrease in LAB population. The optimum water temperature was 40°C.
3. There was interaction effect between palm oil and water temperature on hardness of BEF pellet feed. Based on microbial profile, BEF pellet should be given to animals prior to 7 weeks of storage.

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