The Effect of Packaging Materials Type and Addition of Turmeric (*Curcuma Domestica*) Flour on Calf Starter Pellet to The Total of Fungi, The Existence of Gram Negative Bacteria and *Aspergillus Flavus*

Ranita Dwi Rahmawati¹, Sri Mukodiningsih and Cahya Setya Utama

¹Department of Animal Sciences, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia.

E-mail: rahmawatirania4@gmail.com

Abstract: The purpose of the research was to examined the effect of packaging material and the addition of turmeric levels on the calf starter pellet to the total fungi, the presence gram negatives and *Aspergillus flavus* at 56 days. The method was used the pelleting process. The research used a CRD with factorial pattern (3x2) and 3 replications. The first factor was the addition of turmeric flour (*w/w*) (0%, 0.6% and 1.2%) and the second factor was the type of packaging material (plastic and paper). Data on the total of fungi were analyzed using ANOVA, while data on the presence of gram negative bacteria and *Aspergillus flavus* were analyzed descriptively. The results showed that there was no interaction effect between the first factor and the second factor to the total fungi. Gram negative bacteria were still found on the pellets in all treatments. Whereas *Aspergillus flavus* was only found at the level 0%(plastic) and 0.6%(paper). The conclusion was the addition of turmeric levels in the calf starter pellet who were packaged with plastic and paper have not been able to inhibit the growth fungi and gram negative bacteria, but can inhibit *Aspergillus flavus* at the turmeric flour level of 1.2%.

1. Introduction

Starter feed is solid feed consisting of calf starter (CS) and fiber source feed given to the calf immediately after birth, it’s used to accelerate development of the rumen. Calf starter feed is a concentrated feed made with special formulation, containing of PK 18% and TDN 75%. It’s given as much as 40% of the calf dry matter needs (60% from milk) [1] [2]. CS feed is given to calf until 6 weeks old, because the rumen developed optimally. Calf starter can be produce everyday or prepared first for the needs during calf maintenance. Because of that, CS quality must be maintained, so that there is no loss of quality during storage. Storage will pose a risk of damage to feed physically and microbiologically. Feed damage can be caused by physical conditions, environment, rodents, organizations such as microbes and insects [3].

Fungi and bacteria are organism that can be found in food and feed. Calf Starter is made from grain feed ingredients such as corn, rice brand and soybean meal. Grain feed ingredients are prone to be contaminated with fungi (mold) such as *Aspergillus flavus*. *Aspergillus* can produce a toxin known as aflatoxin. Bacteria that can contaminate feed and endanger livestock are pathogenic bacteria and include in gram negative bacteria. This is very dangerous if contaminated feed is eaten by calf, so it needs control to reduce the growth of fungi and bacteria in the feed. The use of chemicals can interfere with human and animal health. Therefore, additives are needed which can inhibit the growth of fungi and bacteria.

Turmeric is a plant that has many benefits as medicines, dyes, preservatives, herbs, spices and cosmetics [4]. The main content of turmeric is curcuminois, essential oil, starch, bitter substances, resin, protein, cellulose and mineral substances. Curcumin is a phenolic substances that can inhibit growth of microbes by denaturing proteins and damaging cell membranes and turmeric can be antifungal [5].

Packaging influences to maintain the quality of CS feed. Packaging will reduce damage and protect feed from friction impact and contamination caused by the environment [6]. The examples of
packaging materials are paper, plastic, burlap. Packaging made of plastic and paper can maintain a ration water content of less than 14% for 8 weeks.

The purpose of the research was to examine the effect of packaging material and the addition of turmeric levels on the calf starter pellet to the total fungi, the presence gram negatives and Aspergillus flavus at 56 days. The benefit of the research was to provide information to the public that packaging materials and the addition of turmeric flour can inhibit developing microbes.

2. Materials and Methods

2.1. Materials

The materials used in the research for CS feed were turmeric flour, rice bran, mill corn, soybean meal, mineral mix, molasses and aquadest. The tools used were pelleter, basin, thermometer, the tray, measuring cup and spatulas.

2.2. Methods

2.2.1. Formula of calf starter. Formula of calf starter was arranged based the needs of newborn calves, PK 19.61% and TDN 79.10% [7]. The CS pellet manufacturing process was carried out according to the method of pellet with conditioning [8]. Calf starter ingredients were mixed. The next step was conditioning with a temperature 80°C for 20 minutes. Then, the feed was chilled until the temperature drops to 50°C. Feed mixed with turmeric flour. Feed printed using an extruder messin with a hole diameter 5 mm. Pellets dried and packed according to the packaging materials of plastic and paper and stored at room temperature for 56 days.

2.2.2. Experimental design. The experimental design used was a completely randomized design (CRD) 3x2 factorial pattern with 3 replications. The first factor was the addition of turmeric flour to the starter calf (w / w), namely T0: 0%; T1: 0.6%; T2: 1.2%. The second factor was the type of packaging material, transparent plastic material with a thickness of 0.5 mm (J1) and brown paper material with a thickness of 0.7 mm (J2). Each package is filled with 250 grams of calf starter pellets. The parameters observed were total of fungi carried out by total plate count(TPC) methods [9] and the presence of gram negative bacteria identified using gram staining by observing the color and arrangement of bacteria, red gram negative bacteria. The presence of A. flavus was identified through a method of incubated TPC.

2.2.3. Analysis of the data. Data on the total of fungi were analyzed using by ANOVA, if there was influence (P<0,05) a further test was carried out with the Duncan multiple region test. Data on the presence of gram negative bacteria and Aspergillus flavus were analyzed descriptively.

3. Result and Discussion

3.1. Total of Fungi

The average total of fungi was presented in Table 1. Based on the results of statistical analysis of the total of fungi on the calf starter pellets which were added with different levels of turmeric and packed with different types of packaging no significant effect (P> 0.05).

| Turmeric addition (%) | Package   | Total CFU/g |
|-----------------------|-----------|-------------|
|                       | Plastic   | peper       |
| 0                     | 2 x 10^3  | 2.5 x 10^3  |
| 0.60                  | 4 x 10^3  | 2 x 10^3    |
| 1,20                  | 6 x 10^3  | 2 x 10^3    |
| Average               | 3 x 10^3  | 2,2 x 10^3  |

Based on the results of statistical tests on the calf starter pellet, it was found that there was no interaction between the type of packaging and the addition of turmeric levels to the total of fungi on CS pellets after being stored for 56 days in the addition turmeric flour levels T0, T1 and T2. It was
assumed that the concentration of turmeric up to 1.2% still could not inhibit fungal growth, while the fungus continued to grow well. Curcumin can be used well as much as 7.5 – 500 mg/kg of feed [10].

Packaging of calf starter pellets using plastic and paper also had no significant effect (P > 0.05) on the growth of the total of fungi. This is suspected that package with paper and plastik have pores so as to allow air entered and can cause mold growth. When there was absorption of moisture in plastic material evaporation would occur more slowly than paper material. Feed packaged using plastic, evaporation was slower than feed packaged using sacks, cardboard or without packaging material [6]. The total of fungi on pellets was still considered safe because it was still below the safety standard. Total of fungi was considered as one of the criteria for evaluating animal feed, feed was not in accordance with the quality standards of cleanliness if there was a fungus above 3x10^5 CFU/g [11].

3.2. The Presence of Gram Negative Bacteria

The presence of gram negative bacteria was presented in Table 2. Based on the research on calf starter pellets which were package using plastic (J1) and paper (J2) and added with turmeric flour (T0, T1 and T2). Gram negative bacteria and Gram positive bacteria were found in almost all treatments. It’s mean that package with plastic and paper and the addition of the level turmeric flour on the CS pellets could not inhibit the growth of gram negative bacteria.

Table 2. The Presence of Gram Negative Bacteria

| Factorial Combination | U1 | U2 | U3 |
|-----------------------|----|----|----|
|                      | T0 |    |    |
| J1                   | T1 |    |    |
|                      | T2 |    |    |
|                      | T0 |    |    |
| J2                   | T1 |    |    |
|                      | T2 |    |    |

The presence of gram negative bacteria was expected because the duration of storage factor, Feed storage can cause a risk of feed damage caused by physical conditions, environment and organism such as microbes, insects and rodent [3]. Growth of Gram negative bacteria was expected develop during the storage process. This was suspected that package with paper and plastik have pores in order to allow the air entered and can cause mold growth. When there was absorption of moisture in plastic material evaporation, it would occur more slowly than paper material. Evaporation was slower than feed packaged using sacks, cardboard or without packaging material [6].

The presence of Gram negative bacteria in CS pellets had a basil shape. It was suspected that the Gram negative bacteria were the Coliform bacteria group. Coliform bacteria group include basil shape bacteria, gram negative, does not form spores and can ferment lactose by producing gas and acid at 37°C in less than 48 hours [12]. Coliform bacteria in a controlled amount can help the digestive system in livestock, but if excessive can cause disease, especially diarrhea [13].

3.3. The Presence of Aspergillus flavus

The presence of A. flavus was presented in Table 3. Based on the research on calf starter which was stored for 56 days packaged using plastic and paper and added with turmeric flour can inhibit A. flavus but could not inhibit the growth of Candida sp. Asperillus flavus was only found in J1T1U1 and J2T0U3. This is shows that at the addition turmeric flour level at T3 A. flavus did not growth. This was because turmeric is an antifungal that could inhibit fungal growth by inhibiting the growth of fungal mycelium. The content of secondary metabolites contained in turmeric can inhibit fungal growth so that turmeric was used as a fungus controller [14].
Table 3. The Presence of *Aspergillus flavus*

| Factorial Combination | Identification               | U1 | U2 | U3 |
|------------------------|------------------------------|----|----|----|
| J1                     | *Aspergillus flavus*         | -  | -  | -  |
| T1                     |                              |    |    |    |
| T2                     |                              |    |    |    |
| T0                     |                              |    |    |    |
| J2                     |                              | -  | -  | -  |
| T1                     |                              |    |    |    |
| T2                     |                              |    |    |    |

The Calf starter pellets were also overgrown with Candida sp. This was suspected that Candida sp. would grow when the media contains a high carbohydrate sources, protein, fats and vitamins. Media that could be grown with fungi was a medium that contains a high source of carbohydrates and source of nitrogen. The addition of glucose (carbohydrates) would also provide a better chance of growing khamr [15]. Brand could be used as a medium to grow Candida sp [16]. Candida sp. those in the body will cause pathological infections called candidiasis. It can occur due to endogenous or exogenous infections [17].

4. Conclusion

The conclusion is the addition of turmeric levels up to 1.2% in the calf starter pellet who were packaged with plastic and paper have not been able to inhibit the growth fungi and gram negative bacteria, but can inhibit *Aspergillus flavus* at the turmeric flour level of 1.2%.

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