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Effect of adding fermented cabbage waste extract to starter feed pellets on the performance of pre-weaning FH calves

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ABSTRACT

Starter feed accelerates reticulo-rumen development in pre-weaning calves, but proper care is crucial to reduce the risk of diarrhea. This study aimed to evaluate the effect of adding fermented cabbage waste extract (FCWE) to starter feed on calf performance. The experimental design used was a completely randomized design, with 4 treatments (T0: SF without FCWE; T1: SF + 2% FCWE; T2: SF +4% FCWE and T3: SF +6% FCWE) and 5 replications. The materials used were 20 pre-weaned FH calves (initial body weight 39 + 4,35 kg), yellow corn ground, soybean meal, rice bran, molasses, mineral mix, cabbage waste, sugar and salt. Feeding trial was done 8 weeks. Starter feed is given twice a day after milk is given, while forage and water are given ad libitum. The parameters measured were dry matter intake (DMI), average daily gain (ADG), rumen VFA and NH_3. The data DMI and ADG were analyzed with ANOVA and Duncan's test, while rumen VFA and NH_3 were analyzed with descriptive analysis. The result indicated that there was no significant effect of treatment on dry matter intake, but there was significant effect ($P<0.05$) of those on average daily gain. Rumen VFA-NH_3 is in accordance with standards. In conclusions, starter feed with 6% FCWE improves pre-weaning calf performance and is recommended as the best formula.

Introduction

Feeding starter feed to pre-weaned calves (aged 2-6 weeks) can accelerate rumen development (Cunningham, 1992. Starter feed requirements are 40% of the daily dry matter requirement for pre-weaned calves, with 60% of the dry matter requirement coming from milk (NRC, 2001). This starter feed consists of high-quality grains and fiber sources (forage), which can stimulate reticulo-rumen development both chemically and mechanically. Grain or other readily available carbohydrate (RAC) in rumen is fermented and known to produce volatile fatty acid (VFA), especially propionate and butyrate that can stimulate the development papillary. Therefore, a combination of grain feed ingredients and fiber sources can be formulated to produce a complete starter feed that has good quality. The results of research on the biological quality of complete starter feed consisting of a combination of grains, corn fodder and 5% molasses in one-week-old Friesian Holstein (FH) calves resulted in increased reticulo-rumen development (Mukodiningsih *et al*., 2016).

On the other hand, pre-weaned calves often experience diarrhea (Cunningham, 2002). This diarrheal disease contributes to 39% of newborn calf deaths (2-10 days old) (Wudu *et al*., 2008). Therefore, proper care is crucial for calves from birth to weaning. Diarrhea is generally caused by *E. coli* from the environment. To suppress *E. coli* numbers, calves are generally given antibiotics during their rearing. However, if antibiotics are given too frequently, this can cause side effects, including antibiotic residues in milk or meet products. Probiotics can be used alternatively as natural antibiotics. Probiotics are live, non-pathogenic microorganisms that help maintain the balance of intestinal microflora in the digestive tract (Shitandi *et al*., 2007). The use of probiotics in large quantities does not typically have negative effects, as they are considered friendly and safe. *Lactobacillus* sp is a probiotic bacterium as lactic acid bacteria (LAB) that can suppress populations of *E. coli*. Milk from milking in the morning that added 10 ml probiotic can decrease 40% diarrhea problem to new birth calves (Aldana *et al*., 2009). In this regard, it is necessary to look for natural materials that are cheap and easy to obtain that can be used as a source of LAB.

Cabbage (*Brassica oleracea* var. capitata) is a nutrient-rich vegetable containing vitamins and minerals. Cabbage production in Indonesia is around 1,363,741 tons per year, and this production generates significant amounts of waste, estimated at around 5-10% by wet weight. Cabbage leaves naturally contain lactic acid bacteria, such as *Lactobacillus* sp. (including *Lactobacillus plantarum, Lactobacillus delbrueckii, Lactobacillus fermentum*, and *Lactobacillus brevis*), but these are present in small numbers. Fermentation can be used to increase the population of these beneficial bacteria, making fermented cabbage waste a promising source of probiotics. The addition of lactic acid bacteria from fermented cabbage waste extract to starter feed can enhance the benefits of the starter feed, namely that the starter feed also contains lactic acid bacteria. The addition of 6% fermented cabbage waste to the starter feed can increase the number of gram-positive bacteria contained in the starter feed (Mukodiningsih *et al*., 2018). Furthermore, when starter feed is given to calves, it not only stimulates rumen development but also reduces the risk of diarrhea. The development of the rumen is indicated by the normal production of VFA and NH_3 in the rumen. Furthermore, the calves produced are healthy and have daily weight gain that is in accordance with the standard.

Based on this, the research about adding fermented cabbage waste extract on pellet starter feed will be done. The aim of this research was to get the best quality formula for calf starter that contain probiotics with a good performance of calve with indicator dry matter intake, rumen VFA-NH_3 and average daily gain.

Materials and methods

This research included two experiments the experiment was arranged in a completely randomized design with 4 treatments and 5 replications. The treatments were arranged from starter feed (SF) with fermented cabbage waste extract (FCWE). There were T0 (starter feed – without FCWE), T1(starter feed-2% FCWE), T2 (starter feed-4% FCWE) and T3(calf starter-6% FCWE). The starter feed was formulated according to Mukodiningsih *et al*. (2016) to meet the nutrient requirements of calves, with 20% protein and 75% TDN (NRC, 2001). Proximate and starch content were analyzed (AOAC, 2005), and NDF was determined according to Van Soest

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(1994). The required materials included cabbage waste, corn ground, rice bran, soybean meal, molasses, mineral mix, sugar, salt, and forage (corn fodder). The equipment used in the study included knives, digital scales, trays, plastic, tape, labels paper, extruder pellet mill, stove, boiler, digital pH meter, plastic wrapping, oven, incubator, autoclave, measuring cups, sterile petri dish, pipette (1 ml), tube test, spatula, Erlenmeyer, and Quebec colony counter.

This research consisted of two stages. In the first stage, cabbage waste was fermented by chopping it into small pieces, blended, and 6% salt and 6.4% sugar were added. The mixture was then fermented anaerobically for 6 days. In the second stage, starter feed pellets were produced through the following process: the ingredients were weighed and mixed according to the formula, then heated (conditioned) at 80°C for 20 minutes. The mixture was then cooled to 30°C. The next step was molding, and the fermented cabbage waste was added according to the treatment. The pellets were molded to a diameter of 6 mm and dried in an oven at 35°C until they reached a moisture content of approximately 13%. The chemical quality of the pellets is presented in Table 1.

Biological qualities were observed during the feeding trial using 20 cross bread FH calves aged 7-14 days and 39.00±4.35 kg-initial body weight. Calves feeding is 40% CS-L and 60% milk (NRC, 2001) given twice a day at 7:00 AM and 3:00 PM. The starter feed (SF-FCWE) was given 30 min after giving milk, and water and corn fodder were provided ad libitum and changed twice a day (Mukodiningsih *et al*., 2016). Feed intake, rumen VFA and NH_3, average daily gain (ADG) were the parameters observed in feeding trial. Rumen sample was taken from calves aged 3 and 6 weeks. Rumen fluid is collected from the cattle's mouth using a rubber hose. The hose is inserted and pushed slowly until it reaches the rumen. Suction is then applied to collect the fluid.

Dry matter intake and body weight gain are measured every 1 week. The data for dry matter intake and average daily gain were evaluated with analysis of variantbased on Duncan test (Steel and Torrie, 1981). Rumen VFA and NH_3 were analyzed with descriptive analysis (Sugiyono, 2018).

Table 1. Proximate analysis (%) calf starter.

Treatment	Water content	Crude protein	Extract ether	Crude fiber	NNE	Ash	TDN
T0	9.71	14.12	5.89	12.43	59.26	8.7	70.56
T1	10.01	14.58	5.7	13.34	57.93	8.45	69.53
T2	9.99	15.03	7.03	8.8	60.74	8.4	75.23
T3	10.13	14.48	5.73	14.45	57.3	8.44	68.73

Results

The Effect of the treatment on dry matter feed intake (DMI) and average daily gain (ADG) were presend in Table 2. The data in Table 2 indicated that adding FCWE in pellet of starter feed has no significant effect on dry matter intake (DMI). However, the addition of FCWE to the starter feed resulted in a significant effect on the average daily gain of pre-weaned calves. Volatile Fatty Acid (VFA) and rumen NH_3 in this study are shown in Table 3. Volatile fatty acid is short chain fatty acids are the main results of carbohydrate fermentation by microbes in the rumen. Volatile fatty acid production affected the ability of microorganisms to produce the enzyme.

Table 2. Dry matter feed intake and average daily gain of FH Calves.

Treatments	Parameters	
	Dry matter intake (g/day)	Average daily gain (kg/day)
T0	88	0.78^{a}±0.09
T1	103.82	0.71^{a}±0.11
T2	117.79	0.60^{ab}±0.12
T3	109.21	1.03^{b}±0.07

*Superscript on the same column indicate significant difference (P<0.05)

Table 3. Mean of Rumen VFA concentration (Mmol/l).

Added fermented waste cabbage	Rumen VFA concentration	
	21 days old	42 days old
T0	201±46.19	101±11.55
T1	179±84.24	285±84.91
T2	210±79.17	230±28.00
T3	247±43.92	233±56.21

Discussion

In the present study, dry matter intake during research were T0=88 g/day, T1 = 103.82 g/day, T2 117.79 g/day and P3 109.21g/day. Although there was no significant effect on the dry matter intake, T0, T1, T2 and T3 were included in range daily amount solid feed for calves from 50 g to 300g at the age of 3 weeks until 17 weeks (NRC, 2001).

Results in Table 3 showed that the average rumen-VFA FH calves at the age of 21 days old (3 weeks) and 42 days old (6 weeks) experienced an increase in the level of provision of 2%, ie 178 mM became 284.7 mM and 4%, ie 210 to 230 mM, whereas at the level of 6% concentration, VFA level was decreased from 246,7mM to 233.17 mM. Increased VFA was also similar in the study Kristensen *et al*. (2007) that the VFA concentration relatively large at week 4 and week 5. The rumen VFA levels of 6-week-old calves for all treatments were higher than those aged 3 weeks. This indicates that as calves age when fed starter feed, rumen development also increases. According to Cunningham (1992) and NRC (2001), rumen development typically occurs between 2 and 6 weeks of age and is accelerated by the provision of starter feed. In this study, the starter feed contained fermented cabbage waste extract with lactic acid bacteria, which is likely contributed to the increase in beneficial microbes as the calves aged. These microbes ferment feed, producing volatile fatty acids (VFAs), which are indicative of feed fermentation and microbial activity in the rumen (Hamianti *et al*., 2016). The increase in beneficial microbes is further supported by the VFA production data (Table 3), where higher FCWE addition resulted in higher VFA production, with the T3 treatment (6% FCWE) yielding the highest value.

Ammonia is one of the results of protein degradation in the rumen. The concentration of ammonia in the rumen is influenced by many factors, among others, the protein content of the feed and the feed rate of degradation in the rumen. Data on ammonia concentration in the rumen during the study are listed in Table 4.

Table 4. Rumen-NH_3 concentration (mM).

Treatment	Rumen-NH_3	
	21 days old	42 days old
T0 (0%)	24.37±3,81	20.10±6.61
T1 (2%)	27.43±6, 58	36.17±13.75
T2 (4%)	52.60±5,09	55.30±15.42
T3 (6%)	34.25±6,98	21.07±3.96

Table 4 shows that the rumen ammonia concentration of calves aged 21 to 42 days increased in the treatment of adding 2% and 4% fermented cabbage waste, but the ammonia level decreased from 21 to 42 days in the treatment of adding 0% fermented cabbage waste and 6%. The increase in rumen NH_3 with increasing age in treatments T1 and T2, it is suspected that *Lactobacillus* sp contained in T1 and T2 that enter the rumen are able to suppress the growth of harmful microbes. Furthermore, beneficial microbes experience an increase in number and activity, including microbes that produce feed protein digesting enzymes that produce NH_3. However, the NH_3 produced has not been widely utilized as a source of N for their growth.

The decrease in NH_3 production with age in the 0% treatment was due to the absence of *Lactobacillus* sp. entering the rumen. *Lactobacillus* is known to be lactic acid bacterium (LAB) that can inhibit the growth of Gram-negative bacteria (Shitandi at al., 2007). Therefore, the absence of LAB in the T0 treatment is thought to cause a decrease in the number and activity of Gram-positive bacteria, including bacteria that ferment protein sources into NH_3. In the T3 treatment, rumen NH_3 production also decreased with increasing age, and rumen NH_3 production for all ages was lower than that of T2. This could be attributed to the addition of up to 6% fermented waste cabbage, which increased the *Lactobacillus* content in the rumen, which in turn reduced rumen pH, inhibiting rumen microbial activity and growth. According to Dehority and Tirabasso (2004), factors influencing rumen microbial population growth and activity include temperature, pH, buffer capacity, osmotic pressure, dry matter content, and oxidation-reduction potential. However, rumen NH_3 production in all treatments (T0, T1, T2, and T3) met the optimal ammonia production standards for supporting rumen microbial protein synthesis, ranging from 3.57 to 7.14 mM (Sutardi, 1983).

There was significant effect ($P<0.05$) in ADG. T3 treatment produces the highest ADG although dry matter intake is the same. It is predicted that the increasing FCWE (6%) in calf starter is followed by the increased intake of lactic acid bacteria (LAB). It is known that LAB is a source for probiotics, So, calve got T3 are healthier than calves treated with T1 and T2. This is also indicated by the amount of *E. coli* in feces from calve (T3) which was 6.3 x 106 cfu / g and below the standard by Boyd and Marr (1980) that in one gram of feces release *E. coli* bacteria around 109 cfu / g. Finally, calves treated with T3 were more efficient in converting feed into production than T1 and T2 treatments. Average daily gain increased with the addition of FCWE to the starter feed, and T3 produced the highest ADG compared to T0, T1, and T2. It is suspected that, although the DMI results were the same in all treatments, T3 contained the highest number of LAB. This is in accordance with the research of Mukodiningsih *et al*. (2018), who found that the addition of fermented cabbage waste extract in the starter feed as much as 6% resulted in the highest number and diversity of bacteria compared to the addition of 2 and 4%. Furthermore, the increase in the addition of FCWE can stimulate the growth of beneficial bacteria in the digestive tract of calves, especially in the rumen. According to Fardiaz1992) the addition of starter can stimulate the growth of bacteria that are beneficial to livestock and is expected to suppress the growth of pathogenic bacteria such as Salmonella and coliform in the digestive tract. Furthermore, in this study, if this occurs in the rumen, beneficial gram bacteria will also increase, further increasing feed digestibility. This is also indicated by the increase in VFA production, with T3 yielding the highest VFA production (Table 3). This is also indicated by the increase in VFA production, with T3 producing the highest VFA (Table 3). Furthermore, since the feed entering the rumen is mostly concentrate, it is suspected that the fatty acid component in the VFA formed is largely propionate. It is known that propionic acid is a precursor in meat formation. Therefore, treatment T3 resulted in the highest ADG.

Conclusion

Adding of fermented cabbage waste extract on calf starter 6% produced dry matter feed intake and average daily gain , VFA and NH_3 rumen accordance with the standards. Starter feed formula supplemented with 6% fermented cabbage waste extract is the best formula.

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Conflict of interest

The authors have no conflict of interest to declare.

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