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The Effect of Storage Time on the Total Lactic Acid Bacteria and Presence of Gram Positive and Negative Bacteria in Calf Starter Pellet Added with Fermented Cabbage Waste

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ABSTRACT

The aim of this research was to examine the microbiological quality of calf starter pellet added with fermented cabbage waste after stored for 0, 4, and 6 weeks. The materials used in this research consisted of cornmeal, rice bran, soybean meal, molasses, mineral mix, and fermented cabbage waste. This research used Completely Randomized Design with 4 treatments and 3 replications. The mixed calf starter pellet consisted of 100% calf starter and 6% of fermented cabbage waste, and then stored for 0 weeks (P0), 2 weeks (P1), 4 weeks (P2) and 6 weeks (P3). The observed microbial qualities were the total lactic acid bacteria and the presence of gram positive and negative bacteria in the mixed calf starters. The total lactic acid bacteria were analyzed descriptively, while the presence of gram bacteria was analyzed with analysis of variance followed with Duncan's test. The result of this research showed that an increase in storage time would result in lower lactic acid bacteria population, while the gram-positive and negative bacteria was not significantly affected. The research concluded that 6-weeks stored calf starter pellet added with 6% of fermented cabbage waste could maintain its lactic acid and gram-positive bacteria population, while also reducing its gram-negative bacteria population as well.

Keywords: Calf starter pellets, Fermented cabbage waste, Gram-positive and negative bacteria, Total lactic acid bacteria

Introduction

The formulation and feeding of starter feed, which consisted of calf starters and fibers, for the newborn calf is aimed to stimulate the rumen development of the calf. The calf starter is known to provide 40% of daily dry matter requirement for the newborn calf, while the other 60% is consumed from the dairy milk (NRC, 2001). It then becomes important that the feed ingredients for calf starter formulation should have a good quality. The carbohydrate content in feed ingredients should be easily available and fermented by the microbial rumens, which resulted in volatile fatty acids (VFA) production in the form of acetic acid, propionic acid, and butyric acid. The propionic and butyric acid production is important as those acids are known to be able to stimulate rumen and papillae development in the newborn calf. Research showed that calf starter with the addition of molasses as much as 5% would result in a qualified complete calf starter that could stimulate rumen development of Holstein Friesian (HF) calf at the age of 2-6 weeks (Mukodiningsih *et al.*, 2010). In feeding with calf starter, the feed can be given in the form of mash or pellet. Hartadi *et al.* (1990) explained that a pellet is a mass form of feed that formed by compressing through the mold hole mechanically.

On the other hand, the newborn calf still often to get diarrhea which is mainly caused by *E. coli* contamination from the environment. The utilization of probiotics that consisted of lactic acid bacteria to prevent the problem is known to be safer compared than using antibiotics. The lactic acid bacteria are bacteria which capable of utilizing water-soluble carbohydrate to produce lactic acid and decreasing the substrate pH, resulting in an acid environment (McDonald *et al.,* 1991). The acid condition thus inhibits other bacteria growth, especially for bacteria that cannot grow in acid environments such as *E. coli*. The lactic acid bacteria can be found naturally in various vegetables.

Cabbage (*Bassica oleracea L*) is one of the vegetables which massively grow in the upland area and is easily withered, damaged, or decayed. Indonesia had produced 1,363,741 tons of

cabbage in 2015, with 5-10% of the cabbage production were potentially turned to waste (BPS, 2015). Naturally, cabbage has already contained lactic acid bacteria and the population of the lactic acid bacteria could be further multiplied by fermentation. The addition of fermented cabbage waste as much as 6% (w/w) to the calf starter pellet resulted in 8.0 x 10^6 CFU/ml of lactic acid bacteria in (Mukodiningsih *et al.*, 2017a) and showed a good calves performance when given to the pre-weaning calves for 6 weeks (Mukodiningsih *et al.*, 2017b).

Feed formulation that contained bacteria or other microbial cultures required special attention on its microbiological component when stored. Bad storage management would result in the growth of other unwanted microorganisms, which resulted in decreased feed quality (Khalil dan Suryahadi, 1997). Lactic acid bacteria will grow optimally in a substrate with 60% moisture and kept anaerobically. A dormant state, or inhibited grow of lactic acid bacteria could occur when the substrate's moisture is below the requirement of the bacteria. The storage of lactic acid bacteria contained feed can be done by keeping the environment anaerobic (McDonald *et al.*, 1991). Moreover, the presence of lactic acid bacteria can also be maintained by storing in vacuum condition by using laboratory plastic silo (Johnson *et al.*, 2005).

The aim of this research is to examine the different storage time on the total lactic acid bacteria and the presence of gram positive and negative bacteria in calf starter pellet added with fermented cabbage waste. The result of this research would provide information on the storage time which still capable to maintain the lactic acid bacteria population and the presence of gram bacteria on calf starter pellet added with fermented cabbage waste. The hypothesis of this research is that long storage time could maintain the total lactic acid bacteria population and reducing the gram-negative bacteria as well.

Materials and Methods

The research was conducted in the Laboratory of Feed Technology, Faculty of Animal and Agricultural Science, Universitas Diponegoro and in the Laboratory of Microbiology and Health Analyst, Faculty of Nursery and Health, Universitas Muhammadiyah Semarang.

Research instruments

The materials used to produce calf starter pellet were yellow corn, rice bran, soybean meal, molasses, mineral mix, salt, sugar. The starter pellet was then added with fermented cabbage waste, which consisted of cabbage wastes (outer leaf parts), sugar, and salt. The materials used for lactic acid bacteria analysis was MRS (de Man-Rogosa-Sharpe) medium, while crystal violet (Gram A), Lugol's iodine (Gram B), 95% alcohol (Gram C), and safranin solution were used for the gram bacteria staining analysis. The instruments used in this research were digital scale, grinder, pellet mill extruder, autoclave, petri dish, microscope, pipette, test tubes, cotton, aluminum foils, Bunsen burner, knife, basin, conditioner, tray, drier, and instruments for lactic acid bacteria and gram bacteria analysis.

Methods

The research was done by using a Completely Randomized Design (CRD) with 4 different storage time treatments, which were stored for 0 weeks (P0); 2 weeks (P1); -weeks (P2); and 6 weeks (P3), and each treatment were replicated for 3 times. The calf starter pellet mixture was made from 100% calf starter and 6% fermented cabbage waste by following Mukodiningsih *et al.* (2017b), and the calf starter was formulated according to its dry matter (19.62% protein and 79.41% total digestible nutrient) content (Mukodiningsih *et al.*, 2010). The cabbage waste fermentation was done by firstly washed the cabbage waste and cut into ±1 cm size, blended, and added with salt and sugar as much as 6% and 6.4% (w/w) respectively. The cabbage waste mixture was then placed into the silo and tightly sealed in anaerobic condition then fermented for 6 days in room temperature. The calf starter pellet with fermented cabbage waste addition was then made by grinding the fermented cabbage waste with all of the feed ingredients for calf starter until the mixture becomes homogenous. The mixture was then added with molasses at 5% (v/w) and conditioned at 80ºC for ±10 minutes, then reduced to ~35°C. The calf starter mixture was then formed into a pellet by using pellet mill extruder, at 5 mm diameter and 1-1.5 length. The next step was drying the calf starter pellet in an incubator/drying cabinet equipped within and out blower at ~35ºC until the calf starter pellet moisture reduced into ~13%. The dry calf starter pellet was then packed in plastic bags, tightly sealed, and stored in room temperature following the research treatments.

The observed parameters in this research were the lactic acid bacteria population and the presence of gram positive and negative bacteria. The lactic acid bacteria population was analyzed descriptively following Belanche *et al.* (2011), while the presence of gram positive and negative bacteria was identified and scored following Soekarto (1987). The obtained score was then analyzed with analysis of variance to examine the effect of storage time to the presence of gram bacteria, and the analysis will be followed with Duncan's multiple range tests to measure the significance (Steel and Torrie, 1995).

Results and Discussion

The total lactic acid bacteria population

The effect of storage time to the lactic acid bacteria and the presence of gram bacteria in the calf starter pellet added with fermented cabbage waste can be seen in Table 1. The result showed that longer storage time would result in reduced

Table 1. Total lactic acid and gram bacteria population in the calf starter pellets added with fermented cabbage waste during storage

Parameters	Treatments			
	T0	T1	T2	T3
Total lactic acid bacteria (Cfu/g)	8.0 x10^6	4.3 x10^6	0.3x10^6	0.2x10^6
Presence of gram bacteria (score)	4.00	3.33	2.67	2.33

lactic acid bacteria population in the calf starter pellet added with fermented cabbage waste. The average lactic acid bacteria population on each treatment was different, which was 8.0x10^6 CFU/ml in P0; 4.3x10^6 CFU/ml in P1; 0.3x10^6 CFU/ml in P2; and 0.2x10^6 CFU/ml in P3. In the 6-weeks storage time, the lactic acid bacteria population were still shown in the calf starter pellet added with fermented cabbage waste. This indicates that even for 6-weeks storage, the bacteria were still capable to utilize the substrate for its growth, as long as the anaerobic condition and moisture level (~13%) were maintained. However, the lactic acid bacteria population was low as the bacteria couldn't optimally utilize the substrate. McDonald *et al*. (1991) reported that lactic acid bacteria population can be maintained by keeping the anaerobic condition during storage, and the silage can be kept for a long time without damaged. Johnson *et al*. (2005) added that the lactic acid bacteria can be maintained by storing the feed in vacuum lab-scale plastic silo.

Gram-positive and negative bacteria

The analysis of variance showed that the storage time of calf starter pellet added with fermented cabbage waste did not give a significant effect (P>0.05) to the gram-positive and negative bacteria. The obtained average score of gram bacteria in a different storage times were 4.00 in P0; 3.00 in P1; 2.67 in P2; and 2.33 in P3. The result of gram bacteria observation in this research indicates that the calf starter pellet added with fermented cabbage waste still able to provide enough energy for the growth of the gram-positive bacteria even after stored for 6 weeks. In all of the storage time treatments, 2-3 colonies of gram-positive bacteria were seen, while the gram-negative bacteria was 0-1 colony.

The gram-positive bacteria observed in this research had a relatively simple cell wall (rod, bacilli, coccus, and diplococci shaped). These characteristics were seen in the calf starter pellet added with fermented cabbage waste even after stored for 6 weeks. The condition thus indicates that the lactic acid bacteria still existed in the calf starter pellet. Fardiaz (1993) stated that lactic acid bacteria were categorized as gram-positive bacteria, and have a rod shape with short bacilli. The presence of gram-positive bacteria in the calf starter pellet added with fermented cabbage waste with lactic acid bacteria characteristics would inhibit the growth of gram-negative bacteria, which resulted in the reduction of gram-negative bacteria population simultaneously during the storage. The lactic acid bacteria utilize water-soluble carbohydrate in the substrate and produced lactic acid which reduces the substrate pH (McDonald *et al*., 1991). The acid condition thus inhibits other bacterial growth such as gram-negative and decaying bacteria which could defect the feed (Supardi dan Sukamto, 1999).

Conclusions

The research concluded that storing the calf starter pellet added with 6% fermented cabbage waste for 6 weeks could maintain its lactic acid and gram-positive bacteria population. Furthermore, the result of this research also showed that 6-weeks storage could reduce the gram-negative bacteria population.

References

Badan Pusat Statistik (BPS). 2015. Survei Pertanian. Produksi Tanaman Sayuran dan Buah – buahan. Badan Pusat Statistik, Jakarta.

Belanche, A., L. Abecia, G. Holtrop, J. A. Guada, C. Castrillo, G. de la Fuente, and J. Balcells. 2011. Study of the effect of presence or absence of protozoa on rumen fermentation and microbial protein contribution to the chyme. J. Anim. Sci. 89: 4163-4174.

Fardiaz, S. 1993. Analisis Mikrobiologi Pangan. Edisi 2. Raja Grafindo Persada, Jakarta.

Hartadi, H., S. Reksohadiprodjo, dan A. D. Tillman. 1990. Tabel Komposisi Pakan untuk Indonesia. Gajah Mada University Press, Yogyakarta.

Johnson. H. E., R. J. Merry, D. R. Davies, D. B. Kell, M. K. Theodorou, and G. W. Griffith. 2005. Vacuum packing: a model system for laboratory' scale silage fermentations. J. Appl. Microbiol. Biotechnol. 98: 106-113.

Khalil dan Suryahadi. 1997. Pengaruh kandungan air dan ukuran partikel terhadap sifat fisik pakan lokal: kerapatan tumpukan, kerapatan pemadatan tumpukan dan berat jenis. Media Peternakan 22: 1-11.

McDonald, P., A. R. Henderson, and S. J. E. Heron. 1991. The Biochemistry of Silage. 2nd edn. Chalcombe Publication, Centerbury.

Mukodiningsih, S., S. P. S. Budhi, A. Agus, Haryadi, and S. J. Ohh. 2010. Effect of molasses addition level to the mixture of calf starter and corn fodder on pellet quality, rumen development and performance of Holstein-Friesian calves in Indonesia. J. Anim. Sci. Tech. 52: 229-236.

Mukodiningsih, S., C. S. Utama, and P. O. Dewi. 2017a. Handling and using waste cabbage as feed additive on pellet of calf starter and it's effect to microbiology quality. Advance Science Letters. 23: 2589-2590.

Mukodiningsih, S., J. Achmadi, F. Wahyono, E. Pangestu, and S. J. Ohh. 2017b. The biologycal quality of adding fermented waste cabbage as probiotic source to pellet calf starter on calf performance. Proceeding International Conference and Seminar SAADC. http://eprints.undip.ac.id/64573/.

National Research Council (NRC). 2001. Nutrient Requirements of Dairy Cattle. Seventh Revised Edition. National Academic Press, Washington, DC.

Steel, R. G. and J. H. Torrie. 1995. Prinsip dan Prosedur Statistika Suatu Pendekatan Biometrik. PT. Gramedia, Jakarta.

Soekarto, S. T. 1987. Penilaian Organoleptik untuk Industri Pangan dan Hasil Pertanian. Penerbit Bhratara Karya Aksara, Jakarta.

Supardi, I. and Sukamto. 1999. Mikrobiologi dalam Pengolahan dan Keamanan Pangan. Penerbit Alumni, Bandung.