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The Effect of Adding Fermented Waste Cabbage as Probiotic Source to Pellet Calf Starter on Rumen Development and Calf Immune

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I. INTRODUCTION

The reticulo-rumen completely develops both physically and metabolically at birth, which is optimal in 2-6 weeks age; depend on feeding as soon as after birth (Cunningham, 1992; Baldwin *et al*., 2004). Good quality calf starter needs to contain both grain and good quality forage to be physically suitable for the development of reticulo rumen (Baldwin et al., 2004). 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 (Lane et al., 2000). This means calves should be provided with not only adequate amount of fibrous feed but also that of RAC (Morisse et al., 2000). Therefore, a balanced combination of both grain and fiber sources has been feed formulator's concern to make a good quality complete calf starter (CCS). Feeding value CCS that is combination calf starter with corn fodder and 5% molasses for Friesian Holstein (FH) calves start on one week old days can promote rumen development of FH calves. (Mukodiningsih et al., 2010; Mukodiningsih et al ., 2015).

On the other hand, a good maintain is needed for calves after birth until weaned. This is corporate with diarrhea diseases to calve in this period. New born calves (aged 2-10 days) mortality rate caused by diarrhea is 39% (Wudu et al., 2008). Diarrhea is generally caused by *Escherichia coli* from environment and that can cause death. Giving regularly feed containing antibiotic has been reported to reduce the population E. coli, but that has side effect to make residue on milk or meet product. Probiotic bacteria are beneficial bacteria that can suppress populations of harmful bacteria in the digestive tract. Probiotic can be used alternatively as natural antibiotics. Probiotics are living non-pathogenic organisms capable of maintaining the balance of intestinal micro flora in the digestive tract (Shitandi et al., 2007). Beside that giving probiotic can increase serum and antibody IgA, IgM and IgG (Haghighi et al., 2006 ; Panda et al., 2008). The uses of probiotic in large quantities not have a negative effect, because probiotics are friendly and safe materials.

Lactobacillus sp is a probiotic bacterium as lactic acid bacteria 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). Waste cabbage is by product of cabbage's outer shells that have been sorted. Waste cabbage naturally contains lactic acid bacteria and fermentation process can

increase the number of lactic acid bacteria. Fermented waste cabbage is selected as the source of probiotics. The addition of microbial lactic acid bacteria from fermented waste cabbage can enhance calf starter's benefits. Feeding calves with probiotic-rich pellets can optimize rumen calves growth by reducing their potential to catch diarrhea.

Base of this, the research about adding fermented waste cabbage on pellet calf starter will be done. The aim of this research are:

1. to get the best quality formula calf starter that is containing probiotic with a good performance and immune of calve.
2. Produce of pellet calf starter containing probotic .

The result from this experiment will be published on :

1. proceeding international seminar Asian-Ausralasian Association of Animal Production (AAAP)
2. proceeding International confernce on Tropical and Coastal Regian Eco-Developmen (oct 25-27 in Bali)
3. Asian-Ausralasian Journal of Animal Science (AJAS).

This research will be done to continue the research have done before. The Illustration 1 is road map all of the reseach.

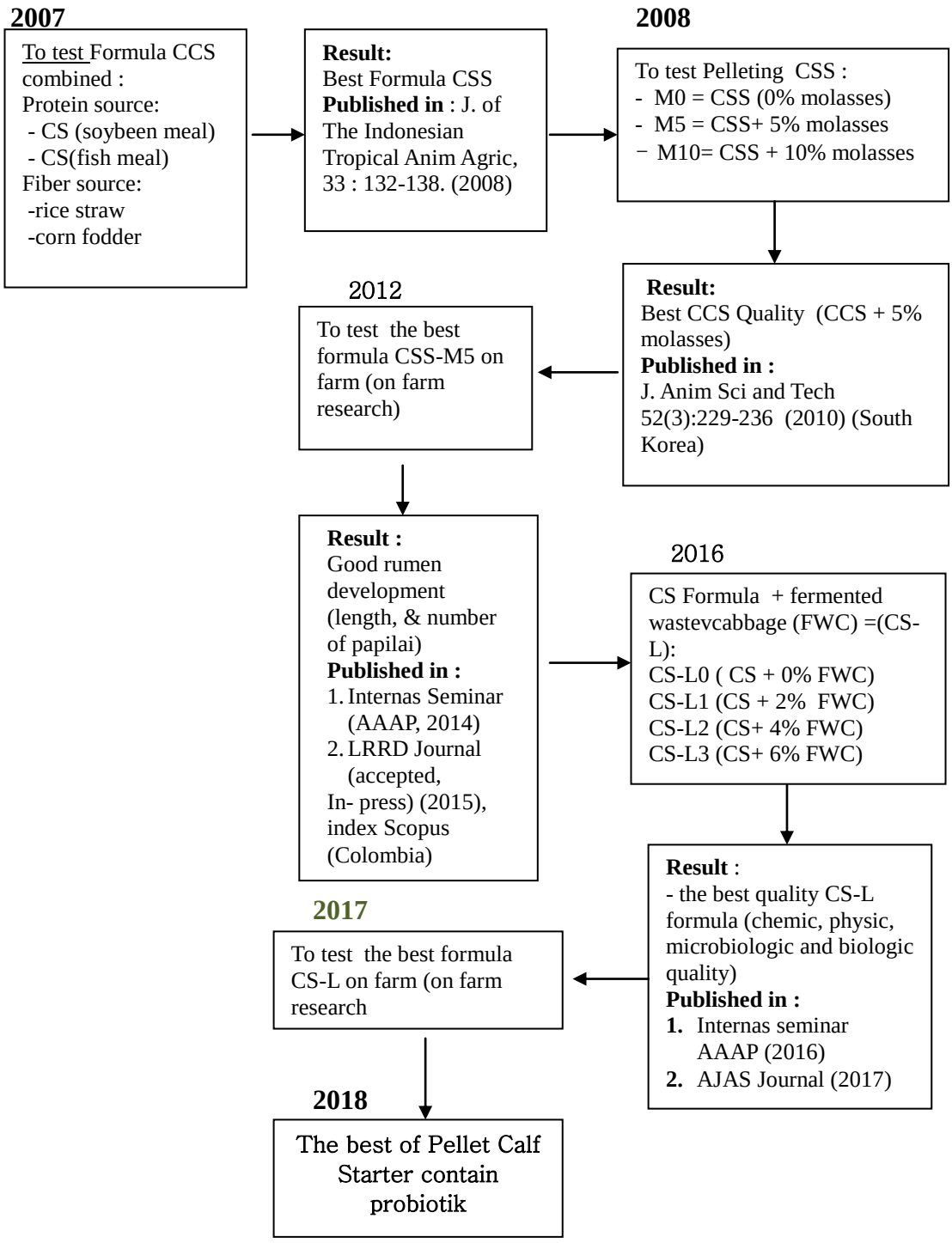


Illustration 1. Road map all of the research

II. LITERATURE REVIEW

Friesian Holstein calf

Friesian Holstein calf is a cross breed between Holstein and Friesian cattle known as dairy cattle type that produces milk highest compared with another cattle. A healthy calf weighs 30 to 35 kg or more at birth. Calves in 3-4 weeks age are growing especially in tructus digestifus. Enlargement of the forestomach occurs rapidly after birth, but the rate is dependent on diet tippy. Beside that, At birth, calve have esophageal groove that functions to divert the flow of ingested milk past the forestomach and into abomasums. So, if calve feeding only milk or milk replacer, forestomach development remain rudimentary for 14-15 weeks or more. Calves can be eating grain and forage at less than 2 weeks old, and are frequently seen to ruminate by 3 weeks of age, indicating considerable forestomach development by this time. The development of the rumen is a critical for weaning and good growth rates after weaning.

Development of the reticulo-rumen

Ruminant are different with monogastric, because ruminant have 4 part stomach that are rumen, reticulum, omasum and abomasum after birth (Church, 1988). The reticulo-rumen is completely developed both physically and metabolically at birth, optimal in 2-6 weeks age, depend on feed that is given as soon as after birth (Cunningham, 1992; Baldwin *et al*., 2004). The reticulo-rumen development is affected by factors, there are: 1. Establishment of bacteria on the rumen; 2. Liquid on the rumen; 3. Outflow of material from the rumen (starter feed) (Quigley, 2001a).

At birth, reticulo-rumen is steril, there are no bacteria present. However by one day of age, a large concentration of bacteria can be found, mostly aerobic bacteria. This bacteria appear to enter on rumen from environment such as saliva or bedding (Cunningham,1992). Than numbers and types of bacteria changes as dry feed enter occurs and the substrate available for fermentation changes. There is a dramatic decrease in the number of aerobic bacteria that occurs approximately 2 weeks after calves begin to consume grain (Cunningham,1992; Quigley, 2001b). This result in a dramatic loss of aerobic and predominant anaerobic bacteria with increasing dry feed intake. Many methanogens, proteolytic, and cellulolytic become established, that are typical rumen bacteria in adult.

Protein and energy is required for calf and that met from the consumption of dry matter derived from milk (60%) and the starter feed (40%) (NRC, 2001). Starter feed consist of a calf starter and a source of fiber or NDF. Therefore, to obtain a good calf growth, should begin 1 week old calf began to be trained or provided calf starter and grass are also provided freely, both in dry form (Cunningham, 1992; Morisse et al., 2000), with a range of 50-250 g / day for aged 3-20 weeks. Calf starter is concentrates or special formula for calves aged 1 week, with crude protein (CP) = 18%, acid detergent fiber (ADF) = 11.6%, neutral detergent fiber (NDF) = 12, 8%, Ca = 0.7% and P = 0.45% (NRC, 2001). The ingredients of calf starter are grains as carbohydrates sources that easily fermented by rumen microbes (readily available carbohydrate/RAC) and protein sources with good quality (Cunningham, 1992). Readily available carbohydrate is fermented by rumen microbe to produce VFA especially propionate and butyrate proportion was higher than acetate, which is very useful to stimulate growth of rumen papillae. Protein sources that are used in the calf starter should have the amino acid composition that resembles the composition of amino acids in milk such as soy bean meal and fish meal. Therefore, increasing the size of particles or feeding with fiber source can maintain the wall of rumen from keratin. According to Cunningham (1992) feed source of fiber mechanical function via friction to maintain the health of the rumen papillae epithelium and formation of keratin which can reduce VFA absorption into the blood. On the other hand, at birth the ability of the rumen to ferment the fiber sources is still low. In this regard, the fiber source given to calf at birth must have good quality such as forage and hay (Lesmeister and Heinrichs, 2005). Feeding value complete calf starter (CCS) that are combination calf starter with corn fodder and 5% molasses for Friesian Holstein (FH) calves start on one week old days have been evaluated (Mukodiningsih et al., 2010; Mukodiningsih et al ., 2013). They found that a combination of calf starter , corn fodder and 5% molasses in pellet form can promote rumen development of FH calves.

Calf immune

The development of calf growth constraints have often experienced that calves are often plagued even death, especially due to diarrhea diseases. Diarrhea disease caused by the bacterium E. coli. Escherichia coli is a disease agent that infects collibacilosis calf weaning the acute and infectious disease transmission. One way to

minimize outbreaks of diarrhea disease is the administration of immune calf . An immune calf, starter calf that has another function as immune . Giving regularly feed containing probiotic, E. coli has been reported to reduce the population in the intestine of calves , thereby improving the health status and lower the cost of treatment of livestock. Beside that giving probiotic can increase serum and antibody immunoglobulin A (IgA), immunoglobulin M (IgM) and Immunoglobulin G (IgG) (Haghighi et al., 2006 ; Panda et al., 2008).

Probiotic

Probiotic is feed supplement from bacteria that can give positive effect to animal with increase balance microflora on tructus digestifus. Probiotic bacteria are beneficial bacteria that can suppress populations of harmful bacteria in the digestive tract of mammals. In addition it serves to replace antibiotics, increase endurance cattle , control and reduce the number of disease-causing bacteria. Lactobacillus sp is a probiotic bacterium that can suppress populations of E. coli. Lactobacillus sp can use of probiotic consisting of cellulolytic bacteria and fungi **.** The use of probiotic in large quantities not have a negative impact , because probiotic are friendly and safe materials. Milk from milking in the morning that added 10 ml pro-biotic can decrease 40% diarrhea dieses to new birth calves (Aldana et al., 2009). Probiotic cultures that contribute to human and animal health through mechanisms such as competition with pathogenic bacteria , stimulates the immune system , increasing the production of short chain fatty acids , control of bowel function , prevent cancer and improve digestion and absorption of nutrients (Ziggers , 2000 ; Jung , et al., 2008) .

Waste cabbage

Cabbage (*Brassica oleracea var. capitata*) is vegetable that contain vitamin and mineral. Cabbage is produced in Indonesia about 1.363.741 ton per year (Badan Pusat Statistika, 2011). Waste cabbage is by product of cabbage's outer shells that have been sorted with number about 5 – 10% wet basis from produce of cabbage. On the cabbage leave, naturally there are bacteria especially lactobacillus sp (*Lactobacillus plantarum, Lactobacillus delbrukil, Laktobacillus fermentum and Lactobacillus brevis*), but in small quantity. Fermentation process can be used to increase the number of lactic acid bacteria.

III. RESEARCH METHOD

This research consist two experiments will be done about 2 year (2016 – 2017). Experiment I (2016) will be conducted to get the best quality of formula calf starter contain probiotic. The best quality of formula from this experiment will be tested on farm in experiment II (2017).

The experiment I (2017) is arranged in completely randomize design with 4 treatments and 5 replication. The treatments are CS-L0 (calf starter − without FCE), CS-L1(calf starter-2% w/v FCE), CS-L2(calf starter-4% w/v FCE) and CS-L3(calf starter-6% w/v FCE). The CS is formulated with ingredients yellow corn ground, rice bran, soybean meal, mineral mix and molasses (mukodiningsih.,et al., 2012) to meet the nutrient requirement of calves with 18% protein and 75% TDN (NRC, 2001). Proximate and starch content were analyzed (AOAC, 1990), and NDF according to Van Soest (1994). The fermented waste cabbage was made from cabbage leave blended and mixed with 6% salt and 6.4% sucrose (w/w), than it should be fermented 6 days. The treatment CS-L is made from CS added FWC with different level and then mixed and pelleted with steam conditioned at 75-80 ˚C for about 15 seconds. The diameter of pellet is ~ 6mm, and then dried until final moisture content ~ 13%. The quality of chemic (proximate and Van Soest), physic (durability and hardness), microbiology (lactic acid bacteria) and biology are parameters that will be observed. The research material included materials and equipment. Waste cabbage, Corn, rice bran, soybean meal, molasses, mineral mix, sugar, salt, de Man Rogosa Sharpe (MRS) medium, Tryptic Soy Broth medium (TSB) and Eosin Methylene Blue Agar (EMBA) medium were the required materials. Equipment used were knives, digital scales, trays, plastic, tape, labels paper, pelleter machine, 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.

The method consisted of two stages. The first stage was fermenting waste cabbage. Waste cabbage was cut into small pieces, blended and added 6% salt and 6,4% sugar and then fermented in anaerobic condition for 6 days. The second stage was making the pellets. Formula calf starter contained 19,62% crude protein and TDN 79,41% (Mukodiningsih et al., 2010). After all the ingredients were mixed, calf starter went through conditioning process at 80ºC temperature for 20 minutes. Before

extruding process, the temperature of calf starter should be decreased at 30ºC temperature and then fermented waste cabbage was added. Pellets were extruded with diameter sized 5 mm. Then, pellets were dried in oven at 34-39ºC temperature to reach13%water content.

The parameters observed were total lactic acid bacteria and *Escherichia coli*. The data of total lactic acid bacteria and *Escherichia coli* were analyzed using descriptive analysis (Belanche et al., 2011).

Biological quality will be observed by feeding trial using cross bread Holstein or Frisian Holstein calves with aged 7-14 days and ± 35 kg-initial body weight. Calves feeding are 40% CS-L and 60% milk (NRC. 2001) are given twice a day at 7:00 AM and 3:00 PM. The starter feed (CS-L) is given 30 min after giving milk (Morisse et al., 2000). Water is provided ad libitum and changed twice a day (Lesmeister and Heinrichs, 2005). Blood ß-hidroxybutirat, blood glucose, blood VFA, rumen development (length, number and tick of papillae), dry matter intake, and calf immune (IgA, IgG , IgM blood content) and body weight gain are parameters observed in feeding trial. Blood and rumen sample will be taken from the calves aged 2, 4, 6 and 8 weeks. Blood sample is taken from vena jugulars about 1 ml. Calves sample are slaughtered for take rumen sample. Dry matter intake and body weight gain are measured every 1 week. All data from first experiment are evaluated with analysis of variant, and it will be continued with Duncan test (Still and Torrie, 1981). The best formula CS-L from the first experiment will be used to second experiment.

The second experiment (2017) will be conducted to test the best formula CS-L (first experiment) in farm. This research will been done for 4 months (pre weaning, weaning and post weaning) using 20 Friesian Holstein (FH) calves (7-14 days old with ± 35 kg initial body weight). Twenty calves are allocated by the completely randomized design during feeding trial. For calves, 40% CS-L and 60% milk (NRC. 2001) are given twice a day at 7:00 AM and 3:00 PM. The starter feed (CS-L) is given 30 min after giving milk (Morisse et al., 2000). Water and forage are provided ad libitum and changed twice a day (Lesmeister and Heinrichs, 2005). Feed intake, daily gain and calf immune (IgA, IgG, IgM blood content) are parameter during the feeding trial study. Calves are weaned gradually, started aged 6 weeks until 8 weeks.

After that, calves are weaned, and feeding ration according NRC (2001) until aged 16 weeks. Feed intake, daily gain and calf immune (IgA, IgG, IgM blood content) are measured during the feeding trial study. The starter feed (CS-L) is given 30 min after giving milk (Morisse et al., 2000). Water is provided ad libitum and changed twice a day (Lesmeister and Heinrichs, 2005). Blood ß-hidroxybutirat, blood glucose, blood VFA, rumen development, (length, number and tick of papillae), dry matter intake, and calf immune (IgA, IgG , IgM) and body weight gain are parameters observed in this experiment. All the data will be evaluated with regression analysis (Steel and Torrie, 1981).

The experiment was done in completely randomize design with 4 treatments and 5 replication. The treatments (T) are calf starter (CS) added fermented waste cabbage (FWC) (w/w):

1. T1 = 100% CS + 0% FWC
2. T2 = 100% CS + 2% FWC
3. T3 = 100% CS + 4% FWC
4. T4 = 100% CS + 6% FWC

For this, more step of the experiment have been done:

1. **Made formula of *calf starter* according NRC (2001) and Mukodiningsih et al (2010) (Table 1)**

Table 1. Formula *Calf Starter* (dry matter basis)

Feed stuff	(%)
Ground corn	43
Rice brand	25,5
Soybean meal	26
Molasses	5,0
Mineral mix	0,5
Nutrient	
- Crude protein	19,62
- TDN	79,41

2. **Proximate analyzed of ingredients were used (Table 2)**

Table 2. Proximate analyzed of ingredients were used

Ingredients	Dry matter	crude protein	Extract ether	Crude fiber	NNE	Ash	TDN**
	---------- (%) ----------						
Yellow corn mill	86,66	9,08	4,61	3,90	81,01	1,40	85,90
Rice brand	93,92	10,57	6,85	14,93	58,46	9,19	70,02
Soybean mill	11,41	49,33	6,35	8,97	27,71	7,64	78,57
Molasses	88,59	3,65	0,26	00,00	86,52	9,57	77,59
Mineral mix		00,00	00,00	00,00	00,00	26,71	00,00

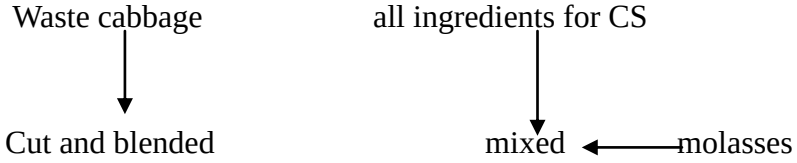
** Rumus Sutardi, 2001.

1. **Pellet calf starter – probiotic has been made**

Pellet calf starter – probiotic was made with two stages (Illustration 2).

- **The first stage made the fermented waste cabbage (FWC).**

Waste cabbage was cut into small pieces, blended and added 6% salt and 6,4% sugar and then fermented in anaerobic condition for 6 day.



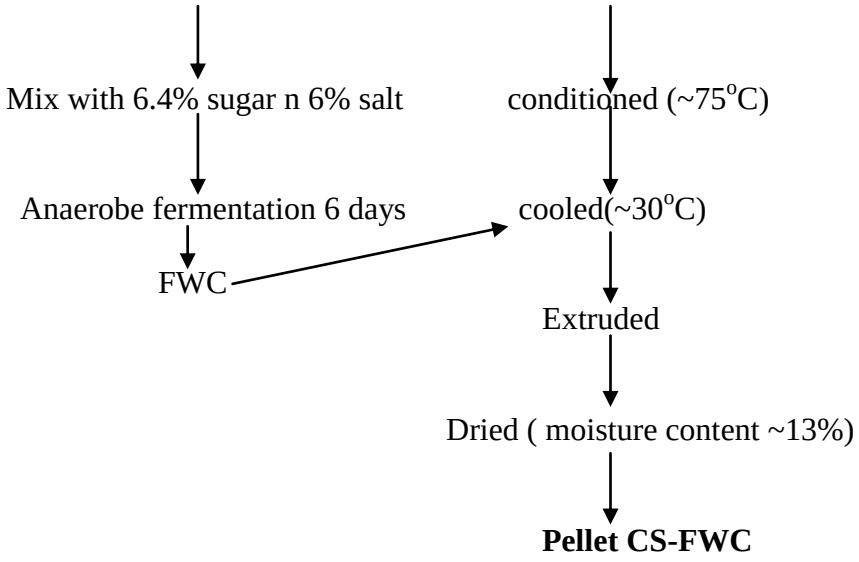


Illustration 2. Diagram process to make pellet of CS-FWC

.

Table 3. proximate analysis from FWC

Water content	Crude protein	Extract Ether	Crude Fiber	NNE	Ash	TDN*
---------- (%) ----------						
34,85	8,79	0,68	4,63	39,5	46,40	4,87

* Rumus Sutardi, 2001.

- **The second stage made the pellet CS-FWC.**

  All the ingredients for calf starter were mixed and added the water 300ml, then conditioned at 80ºC temperature for 15 minutes. Before extruding process, the temperature of calf starter should be decreased at ~ 30ºC and then FWC was added, as the treatment. The mixture were extruded with diameter sized 5 mm. Then, pellets was dried in oven at 34-39ºC temperature to reach 13% water content.

Biological quality have been done by feeding trial using Frisian Holstein calves with aged 7-14 days and ± 35 kg-initial body weight. Feeding trial stared June - August 2016 at Balai Pembibitan Ternak Unggul Sapi Perah dan Hijauan Pakan Baturraden. Calves feeding are 40% CS-FWC and 60% milk (NRC. 2001) are given twice a day at 7:00 AM and 3:00 PM. The starter feed (CS-FWC) was given 30 min

after giving milk (Morisse et al., 2000). Water is provided ad libitum and changed twice a day (Lesmeister and Heinrichs, 2005). Blood ß-hidroxybutirat, blood glucose, blood VFA, VFA and NH3 rumen, dry matter intake, and body weight gain were parameters observed in feeding trial. Blood and rumen sample will be taken from the calves aged 2, 4 and 6 weeks. The dry matter intake and body weight gain have observed, but the analyzed for another parameters have not finished yet.

RESULT AND DISCUSTION

The chemic quality of pellet (CS-FWC)

Table 4. Proximate analysis CS-FWC

Treatment	Water content	Crude protein	Extract ether	Crude fiber	NNE	Ash	TDN*
	---------- (%) ----------						
T0	9,71	14,12	5,89	12,43	59,26	8,70	70,56
T1	10,01	14,58	5,70	13,34	57,93	8,45	69,53
T2	9,99	15,03	7,03	8,80	60,74	8,40	75,23
T3	10,13	14,48	5,73	14,45	57,30	8,44	68,73

Sumber : * Rumus Sutardi, 2001.

Physic-Organoleptic Quality

Average measurement results pellet durability of calf starter was added with fermented cabbage waste can be seen in Table 4.

Table 5. Mean of Physic-Organoleptic Quality to Pellet of *Calf Starter* that added with Microbial Source from Fermented Waste Cabbage

Treatment	Parameter			
	Durability (%)	(Hardness) (kg)	Score Texture	Score of color
T0 (0%)	93,312	2,06	2,28	2,85
T1 (2%)	92,064	1,91	2,56	2,45
T2 (4%)	93,000	1,75	2,31	2,76
T3 (6%)	92,632	1,81	1,85	2,53

Results of analysis of variance showed that the addition of sewage treatment cabbage fermented in calf starter pellet form, showing no significant effect ($p > 0.05$) to the level of durability of pellets. Value durabiltas pellets calf starter in this study were within the range of 92 to 93.3%. This indicates that the value of the durability of pellets calf starter in this study had good durability value. This is in accordance with the opinion of Dozier (2001) which states that the standard specification of durability

index used is the minimum of 80%.

Microbiology quality

Total bacteria

Mean of total bacteria and lactic acid bacteria contained on calf starter pellets during research were shown in Table 5.

The results from the research showed that the average of total bacteria increased with level of addition of fermented waste cabbage. Moreover, the increasing addition of the fermented cabbage waste up to 2% was more reducing the bacterial population. The bacterial population was reduced due to the acidic conditions of the pellet. The addition of the fermented cabbage waste increases, the degree of acidity pellets tend to be increase then the pH value decreasing (T0 =5,71; T1 = 5.76; T2 = 5.65 and T3 = 5.50). This is in accordance with the opinion Utama and Sumarsih (2010), that the addition of sauerkraut extract can accelerate the creation the acidic conditions. The bacteria are not resistant to acidic conditions and it will die, causing bacterial population reduced.

Table 6. Mean of Total Bacteria and Lactic Acid Bacteria on Calf Starter Pellets in Addition with Microbial Source from Fermented Waste Cabbage

Treatment	Total bacteria	Total Lactic Acid Bacteria
	----------cfu/g----------	
T0 (0%)	0.33×10^{11}	0.33×10^{6}
T1 (2%)	0.81×10^{11}	0.60×10^{6}
T2 (4%)	0.53×10^{11}	0.63×10^{6}
T3 (6%)	0.15×10^{11}	0.80×10^{7}

Total lactic acid bacteria and *Escherichia coli* calf starter pellets during research were shown in Table 7.

The treatment of adding microbial source from fermented waste cabbage in calf starter pellets revealed that the increase of fermented waste cabbage addition had consequently increased total lactic acid bacteria (T0: $3,3 \times 10^{5}$cfu/g; T1: $6,0 \times 10^{5}$cfu/g; T2: $6,3 \times 10^{5}$cfu/g; T3:$8,0 \times 10^{6}$cfu/g).

Table 7. Mean of Total lactic acid bacteria, *Escherichia coli* of Calf Starter Pellets that Added Mic with microbial Source from Fermented Waste Cabbage

Treatment	Total Lactic Acid Bacteria	*Escherichia coli*
	----------cfu/g----------	
T0 (0%)	$3,3x10^5$	-
T1 (2%)	$6,0x10^5$	-
T2 (4%)	$6,3x10^5$	-
T3 (6%)	$8,0x10^6$	-

Total Lactic Acid Bacteria

Table 7 showed that lactic acid bacteria could grow and developed properly since the environmental conditions were favorable for them. Lactic acid bacteria's growth was influenced by several factors including: temperature, pH, and nutrient sources (the amount of fermentable carbohydrates or sugars). The addition of molasses in calf starter ration and sugar in fermented waste cabbage was adequate for lactic acid bacteria's nutrient requirement. Molasses contained glucose, carbohydrate and organic acid. Molasses and sugar functioned as carbon and nitrogen sources that were used as energy for bacteria (Richana et al., 2000).Microorganism especially lactic acid bacteria required nutrients such as carbohydrates for survival.

Regarding its acidity, calf starter pellets's pH ranged from 5,50 to 5,76. The temperature in drying oven was 34 – 39^{o}C. These conditions are optimal conditions for the growth of lactic acid bacteria. The optimal temperature for lactic acid bacteria was 25-37^{o}C (Supardi and Soekamto, 1999). Lactic acid bacteria can grow in pH ranged from 3 to 10.5, but the optimal pH range was 5,5-6,0 (Jay, 1996).

Escherichia coli

The results of microbiological analysis showed that *Escherichia coli* were not found in calf starter pellets. *Escherichia coli* grew optimally at pH ranged 7,0 to 7,5. The pH of pellets ranged 5,50 to 5,76. This acidic condition was considered the deciding factor *Escherichia coli* could not grow. Acidic condition inhibited the growth of some types of pathogenic microorganisms. Acidic condition in pollard extract fermented with vegetable waste inhibit gram-negative bacteria such as

Salmonella and *Escherichia coli* to grow, so that the dominant population of microorganism were-gram-positive bacteria (Utama et al., 2013).

Escherichia coli lived in the temperature ranged from 10 to 40°C with an optimum temperature of 37°C. Conditioning process in pellets industry was 80°C during steaming. This condition was lethal for pathogens in feed material. This was in accordance with Parker (1988) which stated that the process of conditioning that aimed to gelatinized also served to reduce the number of gram-negative bacteria or pathogens that might be present in the feed material in pellets production.

Lactic acid bacteria were the reason why *Escherichia coli* was not found. Lactic acid bacteria produced hydrogen peroxide that inhibit decomposing microorganisms through oxidation effect on microorganism's cell membranes. Hydrogen peroxide's activity could damage the cytoplasmic membrane of gram-negative bacteria while gram-positive bacteria were able to survive because of the structure of cell membrane is thicker than the gram-negative bacteria (Muwakhid et al., 2007). Furthermore, Lunggani (2007) added that lactic acid bacteria were capable of inhibiting gram-negative bacteria.

The biological quality

The effect of treatment on the total amount Escherichia coli bacteria in feces

The observation of Escherichia coli on calf feces with feeding pellets calf starter that added fermented waste cabbage is presented in Table 8.

Table 8. Mean of *Escherichia coli* Feces

Treatment	Mean *Escherichia coli* on feces
	---------- (cfu/g) ----------
T1 (2%)	$5{,}9 \times 10^6$
T2 (4%)	$2{,}6 \times 10^6$
T3 (6%)	$6{,}3 \times 10^6$

The results showed that there were no differences among the treatments the number of E. coli, but each treatment has a number of E. coli bacteria that is better than the standard. Boyd and Marr (1980) stated, in one gram of faecal bacteria E.coli issued about 109 cfu / g. E.coli grow well at a temperature of environment in

Indonesia. This is according to Gupte (1996) which states, a growth temperature of E. coli is at a temperature of 37 ° C but can grow at a temperature of 15-45oC. HPT BBPTU Baturraden environment is an environment which is ideal for the growth of bacteria E.col because it has an average temperature during the day and night 24,41oC and 22,22oC as well as daytime and nighttime humidity 80.70% and 82.60%.
The data of dry matter intake and body weight gain :

Blood leucosit

Table 9. mean of blood of leucosit

Minggu ke-	T1	T2	T3
		---------- (/ml) ----------	
3	11.325	11.200	9.800
6	11.850	11.425	11.125

According to the table it can be seen that the calf aged 3 weeks to have a value of leukocytes as follows: T1: 11,325 / ml, T2: 11,200 / ml, and T3: 9.800 / ml. While the value of leukocytes in calves when he was 6 weeks is T1: 11 850 / ml, T2: 11 425 / ml, and T3: 11 125 / ml. However, the value is still within the normal range and in accordance with the statement of Lumsden et al. (1980) that the calf aged 2 weeks - 6 months had a value of 5.6 to 13.7 leukocytes X103 / ml. Bami et al. (2008) suggested that the 7-24 day-old calf had leukocyte counts of 10.92 to 13.88 X103 / ml. Meanwhile, according to (schalm). The number of normal leukocytes is 5.1 to 13.3 X103/ml.

Blood- erythrocytes

Based on the research that has been done shows that the average value of calf blood erythrocytes treated pllet calf starter with the addition of fermented cabbage waste listed in Table 10.

Table 10. Mean of Blood- erythrocytes

Age (week)	T1	T2	T3
		---------- (× 10^6 /ml) ----------	
3	4,62	5,02	4,92
6	4,60	4,93	4,49

Based on Table 2 that in calves aged 3 weeks to have an average value eritrisit as follows: T1: 4.62 million / ml, T2: 5,02juta / ml, and T3: 4.92 million / ml. While the average value of erythrocytes in calf when he was 6 weeks is T1: 4.60 million / ml, T2: 4.93 million / ml, and T3: 4.49 million / ml. The erythrocyte value is less than the stated Choliq (1992) which states that the 0-8 week-old calf has 5.85 to 7.00 million red blood cells / ml and statements Prihantoro et al. (2012) that calves aged 8-14 weeks were inoculated rumen bacteria digesting fiber origin buffalo had red cell count of 6.66 to 8.67 million / ml.

Blood Hemoglobin

Based on the research that has been done shows that the average value of calf blood hemoglobin treated pelleted calf starter plus cabbage fermented waste is contained in Table 11.

Table 11. Mean of Blood Hemoglobin

Age ((weeks)	T1	T2	T3
	---------- (g/dl) ----------		
3	9,98	9,68	9,85
6	9,68	10,00	10,15

The effect of treatment on rumen-VFA

Volatile Fatty Acid (VFA) is short chain fatty acids are the main results of carbohydrate fermentation is carried out by microbes in the rumen. VFA production affected the ability of microorganisms to produce the enzyme. The measurement results in this study are in Table 9.

Table 12 shown that the average rumen- VFA FH calves at the age of 3 weeks and 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 Award VFA suffered lowering namely, 246,7mM be 233.17 mM**.**

Table 12. Rumen-VFA concentration

Treatments	Rumen-VFA concentration	
	3 weeks age	6 weeks age

	----------mM----------	
T1 (2%)	178,667	284,667
T2 (4%)	210	230
T3 (6%)	246,667	233,167

Increased VFA was also similar in the study Rivas (2011), VFA has increased at ages 2 to 8 weeks, whereas at 12 weeks of decline. It is not much different from the research Kristensen et al. (2007), which explained that the VFA concentration peningkata relatively large at week 4 and week 5.

The average yield VFA measurement is too high when compared with the results of research Rivas (2011), calf off colustrum fed calf starter VFA commercial concentrations of 83.78 mM, the value is not much different from the results Santos et al. (2015), that the calf colostrum off without being given the treatment has a concentration of 93.58 mM rumen VFA. VFA concentration in the rumen is influenced by several factors, including feed composition, shape and particle size of the feed. Conrad et al. (1958), explains that the calf starteryang pelleted feed containing fiber can drive the VFA levels in compare forage feed. The particle size and composition of the ration may affect rumen VFA production, feed in pellet form has a particle size smaller rations, so that higher feed Fermentability (Nocek and Kesler, 1980).

The effect of treatment on rumen-NH3

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. The research data concentration of ammonia in the rumen of a biological test results pelleted calf starter with the addition of fermented cabbage waste listed Table 13.

Table 13 shows that the measurement of the concentration of ammonia in calves aged 3 weeks and 6 weeks experienced an increase in the addition of fermented cabbage waste 2% and 4%, while the addition of fermented cabbage waste as much as 6% decline.

Table 13. Rumen-NH3 concentration

Treatment %	Rumen-NH3	
	3 weeks age	6 weeks age
	----------mM----------	
T1 (2%)	27,4333	36,1667
T2 (4%)	52,6	55,3
T3 (6%)	31,2667	21,3667

It is not much different from the research Rivas (2011), that calves fed calf starter increased rumen ammonia from week to week. According to Godfrey (1961) states that the rapid increase in rumen ammonia occurs at the age of 5 weeks and decreased at the age of 17 the following week. The concentration of ammonia in the rumen should decline with the age of the calf, it is because the beneficiary of ammonia better by mikrooraganisme rumen, and rumen volume increases (Anderson et al., 1987).

CONCLUSIONS

In conclusions, the higher addition of fermented waste cabbage the better increasing of total lactic acid bacteria. Moreover *Escherichia coli* were not found in the pellets product. biological test results showed that the addition of calf starter with fermented cabbage waste 6%, resulting in a lower content of E coli feces, blood sugar normal, FVA and NH3 rumen also in accordance with the standards.

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Appendix

Lactic acid bacteria on CS-FWC

Treatment	Replication					mean
	1	2	3	4	5	
	---------- (cfu/g) ----------					
T0 (0%)	-	-	-	$3,3 \times 10^5$	$3,2 \times 10^5$	$3,2 \times 10^5$
T1 (2%)	-	$6,0 \times 10^5$	$6,00 \times 10^5$	-	-	$6,0 \times 10^5$
T2 (4%)	-	$7,4 \times 10^5$	-	-	$5,2 \times 10^5$	$6,3 \times 10^5$
T3 (6%)	-	$7,0 \times 10^6$	$9,10 \times 10^6$	-	-	$8,0 \times 10^6$

Total bacteria CS-FWC

Code	Bacteria population
	---------- (cfu/g) ----------
T0U1	-
T0U2	$1,5 \times 10^{10}$
T0U3	$5,2 \times 10^{10}$
T0U4	-
T0U5	-
T1U1	$8,7 \times 10^{10}$
T1U2	$8,4 \times 10^{10}$
T1U3	-
T1U4	$7,40 \times 10^{10}$
T1U5	-
T2U1	$4,60 \times 10^{10}$
T2U2	$6,10 \times 10^{10}$
T2U3	-
T2U4	-
T2U5	-
T3U1	$1,5 \times 10^{10}$
T3U2	$1,6 \times 10^{10}$

T3U3	$1,5 \times 10^{10}$
T3U4	-
T3U5	-

Identification of spore from bacteria-gram

Wet *Pellet*	Note
T0	+
T1	+
T2	+
T3	+
Dry pellet	**Note**
T0U1	+
T0U2	+
T0U3	+
T0U4	+
T0U5	+
T1U1	+
T1U2	+
T1U3	+
T1U4	+
T1U5	+
T2U1	+
T2U2	+
T2U3	+
T2U4	+
T2U5	+
T3U1	+
T3U2	+
T3U3	+
T3U4	+
T3U5	+

Identification Bacteria-Gram

Score :

Wet pellet	Batang +	Batang Berderet +	Batang Pendek +	Coccus +	Duplo coccus +	Batang -	Batang Berderet -	Batang Pendek -	Coccus -	Duplo coccus -
T0	V	V	-	-	-	V	-	-		-
T1	V	V	-	-	-	-	-	-		-
T2	V	V	-	-	-	-	-	-		-
T3	V	V	-	V	-	-	-	-		-
Dry pellet	Batang +	Batang Berderet +	Batan Pendek +	Coccus +	Duplo coccus +	Batang -	Batang Berderet -	Batang Pendek -	Coccus -	Duplo coccus -
T0U1	V	V	V	-	-	-	-	-	-	-
T0U2	V	V	V	-	-	-	-	-	-	-
T0U3	V	V	-	-	-	-	-	-	-	-
T0U4	-	V	-	-	V	V	-	-	-	-
T0U5	-	V	-	-	-	V	-	-	-	-
T1U1	V	V	V	-	-	-	-	-	-	-
T1U2	V	V	V	-	-	-	-	-	-	-
T1U3	V	V	-	-	-	-	-	-	-	-
T1U4	-	V	-	-	V	-	-	-	-	-
T1U5	-	V	-	-	-	V	-	-	-	-
T2U1	V	V	V	-	-	-	-	-	-	-
T2U2	V	V	V	-	-	-	-	-	-	-
T2U3	V	V	-	-	-	-	-	-	-	-
T2U4	-	V	-	V	V	-	-	-	-	-
T2U5	-	V	V	-	V	-	-	-	-	-
T3U1	V	V	-	-	-	-	-	-	-	-
T3U2	V	V	V	-	-	-	-	-	-	-
T3U3	V	V	V	-	-	-	-	-	-	-
T3U4	-	V	V	V	-	-	-	-	-	-
T3U5	-	V	V	-	V	-	-	-	-	-

- Terdapat 5 jenis bakteri Gram Positif dan 0 jenis bakteri Gram negatif : 7
- Terdapat 4 jenis bakteri Gram Positif dan 0 jenis bakteri Gram negatif : 6
- Terdapat 3 jenis bakteri Gram Positif dan 0 jenis bakteri Gram negatif : 5
- Terdapat 2 jenis bakteri Gram Positif dan 0 jenis bakteri Gram negatif : 4
- Terdapat 1 jenis bakteri Gram Positif dan 0 jenis bakteri Gram negatif : 3
- Terdapat 5/4/3/2/1 jenis bakteri Gram Positif dan 1 jenis bakteri Gram negatif : 2
- Terdapat 5/4/3/2/1 jenis bakteri Gram Positif dan 2/3/4/5 jenis bakteri Gram negatif : 1

Treatment	Start body weight (kg)	Birth	Final body weight (kg)	Time of observed	Daily gain
T0. U2	31.0	31-MEI-2016	62.5	42	0.732558
T0. U1	42.0	01-JUNI-2016	76	42	0.809524
T0. U3	34.0	01-JUNI-2016	69.7	42	0.85
T0. U5	34.0	02-JUNI-2016	70	42	0.857143
T0. U4	38.0	03-JUNI-2016	65	42	0.642857
T1. U1	53.5	09-JUNI-2016	79	43	0.593023
T1. U2	43.0	24-JUNI-2016	73	42	0.714286
T1. U3	30	30-JUNI-2016	53	42	0.547619
T1. U4	35	10-JULI-2016	58	42	0.547619
T1. U5	33.5	10-JUNI-2016	65	42	0.75
T2. U1	40.6	08-JULI-2016	63	43	0.52093
T2. U2	33.0	17-JUNI-2016	63	42	0.714286
T2. U3	49.0	26-JUNI-2016	68.5	43	0.453488
T2. U4	46	01-JULI-2016	75.5	42	0.702381
T2. U5	38.0	09-JUNI-2016	66.5	42	0.662791
T3. U1	35.0	18-JUNI-2016	61.5	42	0.630952
T3. U2	45	06-JULI-2016	72	42	0.642857
T3. U3	33	11-JULI-2016	61	42	0.666667
T3. U4	38.0	23-JUNI-2016	60	42	0.52381
T3. U5	40.0	13-JUNI-2016	71	42	0.738095

Konsentrasi amonia (NH_3)

no pedet	perlakuan	Amonia (NH3)	
		mg3(mM)	Mg 6(mM)
2437	T0U1	23,3	13,4
2436	T0U2	21,2	20,3
838	T0U3	28,6	26,6
846	T1U1	23	51,6
2441	T1U2	24,3	31,7
2442	T1U3	35	25,2
848	T2U1	14	31,3
2440	T2U2	75,5	56,4
847	T2U3	68,3	78,2
2443	T3U1	40,3	18,6
849	T3U2		19,6
2439	T3U3	28,2	25,9

Konsentrasi *Volatile fatty acid* (VFA)

no pedet	perlakuan	VFA	
		mg 3 (mM)	mg 6 (mM)
2437	T0U1	254	94
2436	T0U2	174	94
838	T0U3	174	114
846	T1U1	162	264
2441	T1U2	270	212
2442	T1U3	104	378
848	T2U1	134	250
2440	T2U2	204	198
847	T2U3	292	242
2443	T3U1	274	251,5
849	T3U2	196	170
2439	T3U3	270	278

Kadar glukosa darah

no pedet	perlakuan	glukosa darah mg 3 (mg/dL)	mg 6 (mg/dL)
2437	T0U1	112	83
2436	T0U2	85	22
838	T0U3	91	99
840	T0U4	87	40
839	TOU5	75	74
841	T1U1	91	109
846	T1U2	87	126
2441	T1U3	36	135
2442	T1U4	130	89
842	T1U5	89	92
844	T2U1	92	62
848	T2U2	99	102
2440	T2U3	87	79
847	T2U4	90	89
2438	T2U5	78	50
845	T3U1	89	108
2443	T3U2	68	89
849	T3U3	37	146
2439	T3U4	88	90
843	T3U5	138	145