Nutrient Digestibility and Performances of Frisian Holstein Calves Fed with *Pennisetum purpureum* and Inoculated with Buffalo’s Rumen Bacteria

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ABSTRACT

Buffalo’s rumen bacteria (BRB) are potential in digesting fiber feed. BRB already adapted well with low quality forages and agricultural byproducts. The aim of this study was to determine the effect of buffalo’s rumen bacteria (BRB) consortium inoculated into preweaning Frisian Holstein calves on nutrient digestibility, physiological status, mineral uptake, and blood profile. This study used 14 isolates of bacteria isolated from rumen fluid of four local buffalos. The research units consisted of seven Frisian Holstein calves at two weeks old with the average body weight of 43.6±4.5 kg. Calves were inoculated with 20 mL of buffalo’s rumen bacteria isolates [4.56 x 10^9 cfu/mL] every morning for 10 weeks. The calves were divided into two groups i.e., three calves received bacterial inoculation and four calves without any inoculation. The variables which were analyzed in the preweaning and weaning period were feed intake, digestibility, average daily gain (ADG), feed conversion ratio (FCR), rumen fermentation characteristics, body weight, physiological status, blood profile, and mineral status. Data were analyzed statistically using t-test. The results showed that inoculation of buffalo’s rumen bacteria into Frisian Holstein calves effectively increased feed intake, characteristics of leukocytes and neutrophils, and cobalt (Co) uptake during the weaning period. Inoculation of rumen bacteria improved rumen pH during preweaning and weaning periods. Inoculation of rumen bacteria also had no negative effects on digestibility, feed conversion (FCR), average daily gain (ADG), and physiological status.

Key words: buffalo rumen bacteria, digestibility, physiological status, blood profile, *Pennisetum purpureum*

ABSTRAK

Bakteri rumen kerbau (BRK) merupakan bakteri yang mempunyai potensi tinggi dalam pemanfaatan pakan sumber serat. Bakteri ini telah teradaptasi dengan baik terhadap pakan hijauan dan limbah pertanian yang berkualitas rendah. Inokulasi bakteri rumen kerbau pada pedet memungkinkan rumen berkembang lebih awal dan fermentasi di dalam rumen lebih optimal. Penelitian ini bertujuan untuk mengetahui efektivitas inokulasi bakteri asal rumen kerbau pada pedet Frisian pada kecercahan pakan, status fisiologis, serapan mineral, dan profil darah. Penelitian ini menggunakan 14 isolat bakteri yang berasal dari cairan rumen empat jenis kerbau lokal. Penelitian terdiri atas tujuh ekor pedet prasapih jenis Frisian Holstein berumur dua minggu dengan rataan bobot badan 43,6±4,5 kg. Rumen pedet diinokulasi 20 mL konsorsium bakteri dalam bentuk dadih setiap pagi hari selama 10 minggu dengan cara dicekok menggunakan spoit pada konsentrasi bakteri 4,56 x 10^9 cfu/mL. Tiga ekor pedet diberi inokulan bakteri dan empat ekor sebagai kontrol. Peubah yang dianalisis pada penelitian periode prasapih dan pascasapih antara lain konsumsi pakan, kecercahan, pertambahan bobot badan, konversi pakan, karakteristik fermentasi, bobot badan, status fisiologis, profil darah, dan status mineral. Data dianalisis menggunakan uji-t. Hasil penelitian menunjukkan bahwa inokulasi bakteri rumen kerbau signifikan meningkatkan konsumsi pakan, jumlah leukosit dan neutrofil, serapan cobalt pada pedet umur 12 minggu. Inokulasi bakteri memperbaiki pH rumen selama penelitian dan tidak memberikan pengaruh negatif pada kecercahan, konversi pakan (FCR), pertambahan bobot badan harian (PBBH), dan status fisiologi pedet.

Kata kunci: bakteri rumen kerbau (BRK), kecercahan, status fisiologis, profil darah, *Pennisetum purpureum*

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INTRODUCTION

Problem on the calves rearing program is the slow adaptation of calves to solid and fibrous feeds which causes a slow weaning process. This slow adaptation is related to the undeveloped calf rumen and rumen microorganisms. The main factor affecting the development of rumen, reticulum and omasum is the type of feed given. Grain and forage can stimulate the development of reticulo-rumen. Specific factors that stimulate the development of rumen epithelial are propionate and butyrate (Davis & Drackley, 1998). The presence of rumen microbes in ruminant has an important role in digesting low quality of feed (Sutardi, 1980). The major fiber digesting bacteria are rumen bacteria that have important roles in digesting fiber feed by increasing the solubility of crystalline cellulose (Shinkai & Kobayashi, 2007). Fiber-digesting bacteria belong to the groups of Fibrobacter succinogenes, Ruminococcus flavefaciens and Ruminococcus albus (Sawanon & Kobayashi, 2006; Kumar & Sirohi, 2013).

Fibrous feed is the main energy source for ruminant and has an important role in the development of calf digestive organs (Castell et al., 2013). During digesting the forage, fiber-digesting bacteria will interact synergistically with non-cellulolytic bacteria (Sawanon & Kobayashi, 2006) by breaking the bonds of methoxyl lignocellulose structures and increase the hydroxyl and carboxyl phenolic (Wahyudi et al., 2010). Some microorganisms, including anaerobic bacteria producing cellulose enzymes, can degrade lignin in the cell walls (Asmare, 2014).

Few studies have looked into the effect of inoculated bacteria into the calf rumen. Inoculated bacteria potentially increased rumen development and fermentation. Inoculated bacteria in preweaning period (4 wk) increased total rumen bacteria and feed intake in fourteen weeks old calves (Prihantoro et al., 2012) and also increased feed intake, feed conversion (Frizzo et al., 2010), and body weight at the age of 3 mo (Dezfouli et al., 2007).

Study about the effect of rumen bacteria of buffalo as probiotic on mineral absorption and blood profile in calves was limited. Lee & Salminen (2009) reported that probiotic increased mineral absorption in animals. Probiotic can increase cobalt (Co) uptake on calves at 14 wk of age (Prihantoro et al., 2012). Kinal et al. (2007) suggested that yeast cells also improved the digestibility and absorption of minerals such as phosphorus, magnesium, calcium, copper, potassium, zinc, and manganese. Study about the effect of probiotic on hematolgy was reported in mice and result showed that probiotic increased hematological parameters and lipid profile (Salahudin et al., 2013).

Buffalo’s rumen bacteria consist of Succinichlamidium ruminis, Acetobirio cellulolyticus, Streptococcus sp, Ruminococcus callidus, Prevotella ruminicola, Bacteroides fragilis, Treponema sp (Pandya et al., 2010). The ability of buffalo’s rumen bacteria in increasing nutritional status, physiological parameters, mineral uptake, and hematological profile in calves during preweaning growth period need to be reviewed. The aim of this study was to determine the effectiveness of buffalo’s rumen bacteria inoculated into preweaning Frisian Holstein calves fed with Pennisetum purpureum as a fiber source on nutrient digestibility, performance, physiological status, mineral uptake, and blood profile.

MATERIALS AND METHODS

Animals

Seven Frisian Holstein preweaning calves at two weeks of age with average body weight of 43.6±4.5kg were used. Calves were housed in individual cages with a size of 2.0 x 1.5 m with dry rice straw as bedding. Three calves (45.3±4.2 kg) were given bacteria inoculation and four calves (41.5±4.0 kg) were used as control.

Preparation of Buffalo’s Rumen Bacteria

Fourteen isolates of buffalo’s rumen bacteria (BRB) were isolated from the rumen fluid of four local buffalos that had the ability to degrade fiber source (Prihantoro et al., 2012). Three kinds of rumen fluids were obtained from buffalos raised in Jonggol, West Java and one was obtained from South Jakarta. A 0.1 mL of single culture bacteria stock was refreshed on 5 mL medium consisted of 3.7% Brain Heart Infusion (BHI); 0.05% Cystein-HCl; 0.05% starch; 0.05% glucose; 0.05% cellulobiusa; 0.05% heme; and 0.05% resazurin and incubated at 39°C. About 2% refreshed culture bacteria was inoculated into 250 mL sterile fresh milk and incubated at room temperature (28-32°C) for 60 h until becoming curds (modified from Anandan et al., 1999). The 14 isolated bacteria were homogenized and then were inoculated into the experimental calf.

Inoculation of Buffalo’s Rumen Bacteria into Preweaning Calves

During the study, water and feed were given ad-libitum. Then, all calves were given 4 L of fresh milk/d until the age of 6 weeks, 3 L/d until the age of 7 weeks, and 2 L/d until the age of 8 weeks. All calves were weaned after the age of 8 weeks. Inoculation of BRB was done by inserting 20 mL/d of BRB (4.50x10^7 cfu/mL) at 08:00 h through the mouth by using plastic syringe. The control calves were without any inoculation. Composition and nutrient content of the ration is presented in Table 1.

Data Collection and Analysis

Data of room temperature and humidity were recorded every day at 08:00 and 15:00 h. Feed intake and nutrient digestibility were measured for 7 d at the ages of 8 wk (preweaning) and 12 wk (weaning) by the total collection method. Fermentability of rumen fluid (VFA, NH_3, pH, and bacteria) and hematolgy were also measured. The rumen fluid was withdrawn by using a vacuum pump, whereas blood samples were taken from jugular vein by using syringe. Respiration, heart rate, and rectal temperature were measured every week at 8:00 and 14:00 h.
Table 1. The compositions ingredient and nutrients content of ration (dry matter)

| Ingredients (% dry matter) | Preweaning period | Weaning period |
|---------------------------|-------------------|----------------|
| Corn                      | 45                | 43             |
| Pollard                   | 15                | 16             |
| Soybean meal              | 30                | 14.5           |
| Molasses                  | 10                | 10             |
| *Pennisetum purpureum*    | 0                 | 16.5           |

Nutrient content

| Dry matter (%)    | 84.03 | 84.38 |
| Crude protein (%) | 20.11 | 16.64 |
| Crude fiber (%)   | 4.88  | 9.96  |
| Ether extract (%) | 3.23  | 1.53  |
| Ash (%)           | 8.66  | 5.02  |
| Nitrogen free extract (%) | 47.15 | 51.23 |
| NDF (%)           | 40.19 | 66.73 |
| ADF (%)           | 12.83 | 11.64 |

Minerals

| Ca (%)     | 1.35 | 3.68 |
| Mg (%)     | 0.25 | 0.27 |
| Zn (ppm)  | 75.36 | 98.35 |
| Co (ppm)  | 2.13 | 2.12 |
| P (%)      | 0.25 | 0.34 |

Note: Laboratory of Feed Science and Technology, Department of Nutrition and Technology, Faculty of Animal Science, Bogor Agricultural University (2009)

RESULTS AND DISCUSSION

Nutritional Status of Calves Inoculated with Buffalo's Rumen Bacteria

The averages room temperatures during the study were 24.26±1.40 °C in the morning and 31.98±1.14 °C in the afternoon with a maximum temperature of 33.47±0.88 °C and minimum temperature of 23.33±1.28 °C. The minimum humidity was 49.57±8.10% and a maximum humidity was 87.24±4.48%. These conditions were not in comfort condition. The ideal humidity should be less than 76%. The conditions of thermo neutral zone for dairy calf are 25-26 °C (Ulvshammar, 2014).

Feed intake and nutrient digestibility in calves inoculated buffalos rumen bacteria are shown in Table 2. There was no difference in dry matter intake between the inoculated calves and control during the preweaning period. Intakes of dry matter, organic matter, crude protein, crude fiber, fat, nitrogen free extract (NFE), and total digestible nutrient (TDN) in inoculated calves during weaning period were significantly higher (P<0.01) than in control calves. This indicates that the inoculation of BRB can improve nutrient intake by improving rumen activity. Inoculation of bacteria in the calf can increase feed intake (Frizzo et al., 2010). Probiotic supplementation increased feed intake (Desnoyers et al., 2009) because it improved cellulolytic bacteria in the rumen (Wallace & Newbold, 1993).

Digestibility of dry matter was not significantly different between inoculated calves and control calves. The nutrient digestibility values of both groups were higher than 74%. The high nutrient digestibility in both groups was an indication of normal growth of rumen microbial population that was able to degrade the feed consumed. Davis & Drackley (1998) revealed that calves were able to consume dry feed at the age of two weeks and Sutardi (1980) suggested that digestibility of the feed was influenced by the quality and type of material fed to the cattle.

Digestibilities of crude fiber in preweaning and weaning calves (Table 2) were not significantly different between the inoculated calves and control calves because the rumens of control calves have evolved. Furthermore, the increase of feed intake at weaning period resulted in the faster retention time and furthermore the decreased digestibility. According to Dias et al. (2011) retention time of digest has a positive correlation with digestibility of the feed. Davis & Drackley (1998) reported that feed intake of calf at ten weeks old is 2.0-2.5 kg DM/d.

Feed conversion ratio (FCR) and average daily gain (ADG) on preweaning and weaning periods of the two groups were not different significantly. Inoculation of bacteria increased average daily gain of calves by 19.47% in weaning period. Yaqub et al. (2004) reported that addition of *Lactobacillus acidophilus* had no significant effect on growth performance and health of calves. Buffalo’s rumen bacteria relatively improved feed conversion ratio (FCR) by 16.89% on weaning period. It indicated that feed intake of calves was efficient. Swinney-Floyd et al. (1999) stated that inoculation of strain *Propioni bacterium*...
P-63 and *L. acidophilus* strain LA5345 on single and mixed conditions of the cattle did not affect FCR.

### Rumen Fermentation Characteristics

Total VFA, NH$_3$, and the number of bacteria in the calves’ rumen fluid were not significantly different between the two groups (Table 3). The average total VFA and the number of bacteria in the calves’ rumen fluid were higher in the inoculated calves as compared to control calves. Rumen pH in the inoculated calves was significantly higher ($P<0.05$) than control calf. This indicates that the BRB inoculation can improve rumen conditions (Musa et al., 2009). Khalid et al. (2011) stated that probiotic increased the rumen pH and improved rumen microbial ecology in calf. The range of rumen pH of calves 3 h after feeding is 6-7.3 (Stobo et al., 1966).

### Calf Performances

The feed intake positively correlated to body weight and calf abdominal circumference (Figure 1). It means that the feed intake is significantly ($P<0.01$) affected by calf body weight and abdominal circumference. This was due to the calves were on the growth phase. Feed intake will increase with calf age (Davis & Drackley, 1998) and inoculation of bacteria to the calf can increase feed intake and feed conversion (Frizzo et al., 2010). The slope rate of feed intake, body weight, and daily body weight gains of inoculated calves were lower than control (Table 4). However, the slope regression of the feed intake of inoculated calves increased more rapidly than control, especially during the weaning period. It showed that the addition of BRB had a positive impact on nutrient intake which was presumably due to the improvements of rumen fermentation system, such as rumen pH. The increased rate of abdominal circumference with

### Table 2. Feed intake and nutrient digestibility in calves inoculated with buffalo’s rumen bacteria

| Parameter               | Preweaning | Control | Weaning | Control |
|-------------------------|------------|---------|---------|---------|
| Calf (n)                | 3          | 4       | 3       | 4       |
| Intake (g/d):           |            |         |         |         |
| Dry matter              | 960.03±129.32 | 738.66±253.62 | 1374.19±12.05$^a$ | 1332.00±6.96$^a$ |
| Crude protein           | 229.75±30.95 | 173.23±66.84  | 270.99±2.38$^a$  | 262.67±1.37$^a$  |
| Crude fiber             | 55.75±7.51  | 42.04±16.22  | 162.21±1.42$^a$  | 157.23±0.82$^a$  |
| Fat                     | 36.90±4.97  | 27.82±10.73  | 24.92±0.22$^a$  | 24.15±0.13$^a$  |
| NFE                     | 538.68±72.56 | 406.16±156.70 | 834.32±7.32$^a$ | 808.70±4.23$^a$ |
| Organic matter          | 861.09±115.99 | 649.25±250.49 | 1292.43±11.34$^a$ | 1252.75±6.55$^a$ |
| TDN                     | 722.73±103.06 | 545.12±214.68 | 1055.68±10.57   | 1042.49±13.53   |
| Digestibility (%):      |            |         |         |         |
| Dry matter              | 77.24±1.24  | 78.08±1.73  | 77.87±1.00     | 79.34±0.83     |
| Crude protein           | 84.48±5.14  | 81.66±2.51  | 77.80±1.31$^b$ | 80.11±1.04$^b$ |
| Crude fiber             | 18.23±4.30  | 21.70±6.01  | 44.41±1.97     | 43.33±4.93     |
| Fat                     | 90.84±3.89  | 85.25±10.98 | 82.96±0.89     | 84.63±1.59     |
| NFE                     | 82.57±1.47  | 82.88±2.80  | 87.06±0.61     | 88.78±1.37     |
| Organic matter          | 77.61±0.95  | 77.85±1.91  | 77.92±0.88     | 79.55±1.15     |
| ADG (g/d)               | 357.15±94.49 | 366.07±110.56 | 565.48±54.55   | 455.36±106.65  |
| FCR                     | 1.78±0.35   | 1.40±0.43   | 2.62±0.19      | 3.15±1.06      |

Note: Means in the same row with different superscripts differ significantly ($P<0.01$). NFE= nitrogen free extract; FCR= feed conversion ratio (FCR); ADG= average daily gain.
the increased feed intake showed that the increase in calf weight could be stimulated by increasing feed intake. The growth of the calf could be improved, because the optimum consumption of the calf has not been achieved. The increase in abdominal circumference over consumption also showed the increased activity of the rumen microbes. Calf ration is generally consisted of grain that contains a lot of simple carbohydrate structures that are easily fermented into propionate and butyrate that can stimulate the growth and development of calf's rumen (Suarez et al., 2006).

Physiological Status

Inoculation of BRB did not affect respiration rate and heart rate in two groups of calves during preweaning and weaning periods (Table 5). It showed that the physiological conditions of the experimental calves were in the normal condition. Davis & Drackley (1998) reported that respiration rate of calves ranged 22-45 breath/min.

Average rectal temperature in the morning during the preweaning period in the inoculated calves was significantly (P<0.05) lower than the control (Table 5). This result indicated that the inoculation of BRB improved the status of the calf's body temperature, although both groups were in the normal range. This result was supported by the data that respiration rate of inoculated calves was lower than control. Davis & Drackley (1998) stated that the normal range of rectal temperature of the calf is 38-39 °C. It showed that inoculation of BRB could change the pattern of rumen fermentation, fermentation products, and body metabolism especially body temperature in the morning.

Blood Profile

Levels of erythrocytes, hematocrit, and hemoglobin of inoculated calves were similar to those of the control. The values of erythrocytes, hematocrit, and hemoglobin were 7.73-8.45 million/mm³, 29.18%-31.66%, and 9.66-10.44 g%, respectively (Table 6). These results indicated that the blood characteristics of both calves groups were in normal physiological conditions. Smith & Mangkoewidjojo (1988) stated that normal levels of hemoglobin, erythrocyte, and hematocrit are 9-15 g%, 29%-35%, and 4.0-12.0 million/mm³, respectively.

Leukocytes of inoculated calves during weaning period were significantly higher (P<0.01) than those of control. This result indicated that inoculation of BRB was able to increase the level of leukocytes. This study showed that level of leukocytes during preweaning and weaning periods were in the normal level. Normal levels of leukocytes were 4.30-14.80 thousand/mm³ at 1-4 wk old and 4.91-14.6 thousand/mm³ at 5-8 wk old (Jezek et al., 2011). Leukocytes are part of immune system that plays a role in regulating the immune system in suppressing pathogen infection (McCowen & Bistrian, 2003).

The results showed that the differentiation of calf leukocytes in normal range was 16.00%-38.25% for neutrophils, 57.00%-78.00% for lymphocytes, 2.33%-4.25% for monocyte, and 0.5%-3.5% for eosinophils. Smith & Mangkoewidjojo (1988) stated that the normal range of neutrophils, lymphocytes, monocytes, and eosinophils of calves were 17.5%-50%, 50%-75%, 0%-6%, and 0%-8%, respectively. Neutrophils from inoculated calves during weaning period were significantly higher than control. Phagocytosis provides a first line of defense against in-

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**Table 3.** The characteristics of rumen fermentation of calves inoculated buffalo’s rumen bacteria

| Parameter | Preweaning | Weaning |
|-----------|------------|---------|
| Calf (n)  | Inoculated | Control  | Inoculated | Control  |
|          | 3          | 4        | 3          | 4        |
| T-VFA (mM) | 138.3±65.38 | 105.00±61.91 | 143.33±56.86 | 110.00±16.32 |
| NH₃ (mM)  | 10.03±1.53  | 7.53±2.47  | 5.68±1.37  | 5.66±2.46  |
| pH        | 7.20±0.33   | 6.64±0.19  | 6.97±0.19  | 6.59±0.19  |
| Bacteria (log cfu/mL) | 13.54±0.18 | 13.24±0.22 | 13.93±0.47 | 13.79±0.29 |

Note: Means in the same row with different superscripts differ significantly (P<0.05).

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**Table 4.** Regression relationship between feed intake, body weight, and average daily weight of calves

| Parameter | Feed intake | R² | Control | R² | P value |
|-----------|-------------|----|---------|----|---------|
| Calf (n)  | 3           |    | 4       |    |         |
|          | Inoculation |    | Control |    |         |
| Preweaning | Y=241.0x + 7.048 | 0.996 | Y=206.5x + 124 | 0.982 | 0.630 |
| Weaning   | Y=138.3x + 1007 | 0.626 | Y=71.69x + 1075 | 0.412 | 0.105 |
| Body weight | Preweaning | 0.986 | Y=3.014x + 37.78 | 0.987 | 0.172 |
|          | Weaning | 0.094 | Y=0.65x + 60.37 | 0.804 | 0.262 |
| Average daily weight | Preweaning | 0.156 | Y=58.16x + 254.7 | 0.434 | 0.536 |
|          | Weaning | 0.438 | Y=-185.7x + 714.2 | 0.62 | 0.544 |

Note: R=correlation coefficient, Sign= significance of the slope among the inoculation with the control group.
vading microorganism, such as cells neutrophils, eosino-
phils and monocyte (Parham, 2009). Increased levels of
neutrophils in the treatment indicated that inoculation
of BRB increased the ability of the calf in response to
foreign substances and pathogens.

**Mineral Status**

The absorption of minerals Ca, P, Mg, Zn, and Co in
both groups of calf is presented in Table 7. Absorptions
of minerals Ca, P, Mg, Zn, and Co in the inoculated
calves were not different as compared to those of control
calves. Absorptions of mineral Ca, P, and Co were more
than 83%. The pattern of mineral absorption tended to
increase during the weaning period. The high absorption
of minerals indicated that mineral needs of the calves
were very high during the growth phase. Darmono
(2007) states that Ca, Mg, Na, K, and P are required to
synthesize the structure of the body, such as bones and
teeth, whereas Fe, Cu, Zn, Mo, and I elements play a
role in facilitating the activity of enzyme and hormonal
systems. On the weaning period, Co absorption in the
inoculated calves was higher (P<0.01) than control. This
indicated that the inoculation of BRB increased the Co
uptake which was possible due to the high synthesis of
cyanocobalamin in the rumen bacteria of the inoculated
calves (Table 3).

The increased uptake of Co was not related to
erthrocytes and hemoglobin levels of calf in each
treatment group, although erythrocytes and hemoglobin
level of inoculated calves were higher than those of
control (Table 6). Wang et al. (2007) suggested that Co
supplementation by 0.086 mg Co/kg DM significantly
increased level of hemoglobin in lamb. Supplementation
of 0.05-1.0 mL Co/kg in fresh feed increased vitamin B
12 in rumen, and it played a role in formation of hemoglo
bin (Tiffany et al., 2006).

The level of mineral in the plasma is a reflection
of the amount of minerals that absorbed through blood
vessels and its value depends on the mineral content in
the feed and the rate of absorption (Girindra, 1988). The
results showed that the concentrations of P and Mg in
the blood were in the normal range, but the levels of Ca
during weaning period in both of groups were below
the normal range whereas the percentage of uptake was
above 94%. This suggested that Ca was needed highly
during the weaning period and the Ca content of the
ration was lower than that required by the calf. Plasma
mineral levels were not different significantly between
the two groups. This showed that the inoculation of

| Parameter                  | Preweaning | Weaning |
|----------------------------|------------|---------|
|                            | Inoculated | Control |
|                            | Inoculated | Control |
| Calf (n)                   | 3          | 4       |
|                            | 3          | 4       |
| Respiration rate (breaths/min) |           |         |
| Morning                    | 39.33±3.91 | 42.29±0.81 |
| Afternoon                  | 48.17±4.76 | 53.83±6.20 |
| Heart rate (beats/min)     |            |         |
| Morning                    | 82.00±1.17 | 83.25±3.07 |
| Afternoon                  | 89.61±1.00 | 91.00±3.23 |
| Rectal temperature (°C)    |            |         |
| Morning                    | 38.94±0.16 | 39.15±0.04 |
| Afternoon                  | 39.44±0.15 | 39.53±0.05 |

Note: Means in the same row with different superscripts differ significantly (P<0.05).
bacteria did not interfere with minerals level of blood and the calves were well utilized the mineral feed. The normal levels of P, Mg, and Ca in the blood of dairy cattle were 3.7-10.1, 1.7-4.3, and 3.7-12.0 mg/100 mL, respectively (Krause et al., 1968).

**CONCLUSION**

Inoculation of buffalo rumen bacteria into Frisian Holstein calves was effective to increase feed intake, characteristics of leukocytes and neutrophils, and Co uptake during the weaning period. Inoculation of rumen bacteria improves rumen pH during preweaning and weaning periods. Inoculation of rumen bacteria has no negative effects on digestibility, feed conversion (FCR), average daily gain (ADG), and physiological status of calves.

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**REFERENCES**

Asmare, B. 2014. Biotechnological advances for animal nutrition and feed improvement. World J. Agric. Res 3:115-118. http://dx.doi.org/10.12691/wjar-2-3-5

Anandan, S., A. Dey, S. M. Deb, S. Kumar, & P. C. Harbola. 1999. Effect of curds as probiotic supplement on performance of Cheghu crossbred kids. Small Rumin. Res. 32:93-96. http://dx.doi.org/10.1016/S0921-4488(98)00159-X

[AOAC] Association of Official Analytical Chemist. 1990. Methods of Analysis of the Association of Analytical Chemist. 16th Ed. Assoc. Off. Anal. Chem., Arlington.

Campbell, C. M. & L. James. 2009. Atomic absorption spectrophotometric and ethylenediaminetetraacetate–titration methods for calcium and magnesium determinations. J. Dairy Sci. 52: 121–124.

Castells, L. A. Bach, A. Aris, & M. Terre. 2013. Effects of forages provision for young calves on rumen fermentation and development of the gastrointestinal tract. J. Dairy Sci. 96:5226-5236. http://dx.doi.org/10.3168/jds.2012-6419

Conway, E. J. 1950. Microdiffusion. Analysis and Volumetric Error. (2nd Ed.). Crosby Lockwood and Son, London.

Darmono. 2007. Penyakit defisiensi mineral pada ternak rumian dan upaya pencegahannya. J. Litbang Pertanian. 26: 391-404.

Davis, C. L. & J. K. Drackley. 1998. The Development, Nutrition, and Management of the Young Calf. Iowa State Press. Iowa.

Desnoyers, M., S. Giger-Reverdin, G. Bertin, C. Duvaux-Pontier, & D. Sauvant. 2009. Meta-analysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. J. Dairy Sci. 92:1620-1632. http://dx.doi.org/10.3168/jds.2008-1414

Dezfouli, M. R. M., P. Tajik, M. Bolourchi, & H. Mahmoudzadeh. 2007. Effects of probiotics supplementation in daily milk intake of new born calves on body weight gain, body height, diarrhea occurrence and health condition. Pak J. Biol. Sci. 10: 3136-3140.

Dias, R. S., H. O. Patino, S. Lopez, E. Pates, K. C., Swanson, & J. France. 2011. Relationship between chewing behavior, digestibility, and digesta passage kinetics in steer fed oat hay at restricted and ad libitum intake. J. Anim. Sci. 89:1873-1880. http://dx.doi.org/10.2527/jas.2010-3156

Frizzo, L. S., L. P. Soto, M. V. Zbrun, E. Bertozzi, G. Sequiera, R. Rodriguez Aimesto, & M. R. Rosmini. 2010. Lactic acid bacteria to improve growth performance in young calves fed milk replacer and spry-dried whey powder. J. Anim. Feed. Sci. Tech. 157:159-167. http://dx.doi.org/10.1016/j.anifeedsSci.2010.03.005

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Table 7. Absorption and mineral status in calves inoculated with buffalo’s rumen bacteria

| Parameter          | Preweaning Inoculated | Control | Weaning Inoculated | Control |
|--------------------|-----------------------|---------|--------------------|---------|
| Calf (n)           | 3                     | 4       | 3                  | 4       |
| Absorption (%)     |                       |         |                    |         |
| Ca (g/d)           | 11.31±2.30            | 8.60±3.51| 48.40±1.82         | 46.49±1.04|
| P (g/d)            | 2.03±0.37             | 1.57±0.54| 4.19±0.07         | 4.00±0.08|
| Mg (g/d)           | 1.43±0.36             | 1.05±0.35| 2.10±0.14         | 2.26±0.42|
| Zn (mg/d)          | 46.48±17.76           | 32.67±10.92| 124.32±2.09   | 121.99±2.38|
| Co (mg/d)          | 2.00±0.28             | 1.55±0.53| 2.86±0.03        | 2.78±0.02|
| Plasma mineral (mg/100mL) |               |         |                    |         |
| Ca                 | 86.40±6.29            | 83.96±12.37| 95.95±3.48         | 94.64±1.66|
| P                  | 84.85±5.21            | 86.07±2.49| 90.53±2.46         | 89.18±1.94|
| Mg                 | 59.47±7.11            | 57.44±5.69| 59.12±3.24         | 62.42±6.24|
| Zn                 | 62.89±17.50           | 59.66±10.71| 91.98±1.03        | 93.12±1.69|
| Co                 | 98.16±0.55            | 98.65±0.42| 98.33±0.16        | 98.5±0.20|

Note: Means in the same row with different small superscripts differs significantly (P<0.05); means in the same row with different capital superscript differs significantly (P<0.01).
