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The effect of different milk replacers on the physiological vital signs, fecal characteristics, and *E.coli* bacteria counts in calves

E.Y. Setyowati[1] , **F.A. Satrio**[2] , **S. Septiyani**[2] , **S. Windria**[2] and **M.F. Wiyatna**[1]

[1]Department of Livestock Production, Faculty of Animal Husbandry, [2]Department of Biomedical Science, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia

Abstract

This study aims to determine physiological vital signs, fecal characteristics, and the number of *E.coli* bacteria in calves given different types of milk. A total of 15 male calves with a body weight of around 40-50 kg were used in this research. The calves were assigned to three treatment groups: LK, commercial, or fresh milk. Physiological vital signs and fecal characteristics were evaluated every two days in the morning for 30 days. Physiological vital signs were assessed based on heart rate, respiratory rate, and rectal temperature. Sampling for the number of *E.coli* bacteria was carried out on days 1, 15, and 30. The feces samples were cultured on EMB agar media using the pour plate method. The results showed that the LK milk group had the same heart rate, respiratory frequency, and temperature as the other groups. All treatments resulted in fecal colours ranging from brownish to yellowish. LK milk exhibited a fecal colour significantly closer to yellowish than commercial milk but was more similar to fresh milk, which tended to be brownish. Fecal consistency was similar across all treatment groups, ranging from soft to runny, with no significant differences. Additionally, the type of milk had no significant effect on the number of *E.coli* bacteria observed throughout the study period. Thus, differences in the type of milk given to calves do not affect physiological vital signs, fecal characteristics, and the number of *E. coli* bacteria in feces.

Introduction

Calf rearing is a critical aspect of the dairy and beef industries, as calves' health and development significantly impact the production system's profitability and longevity (1). The transition from milk to solid feed during the weaning process of dairy calves can be a challenging period marked by heightened stress and increased susceptibility to infectious diseases (2). Milk replacers are frequently used as a cost-effective alternative to whole milk, impacting calves' physiological well-being, growth, and overall performance (3,4). Choosing an appropriate milk replacer is crucial, as different formulations can influence a calf's development, including its physiological vital signs, fecal characteristics, and susceptibility to pathogens like *Escherichia coli*. Vital signs like body temperature, heart rate, and respiratory rate are key indicators of a calf's health and can be affected by the composition and quality of the milk replacer. In addition, the characteristics of a calf's feces—such as consistency, colour, and abnormal elements—can offer important information about the animal's digestive health and how different milk replacers may affect it. Additionally, the presence and quantity of *E. coli* bacteria in the calf's digestive system can significantly affect their overall health and well-being, as this pathogen can cause severe gastrointestinal issues if not properly managed (2).

This study investigated the effects of different milk replacers on the physiological vital signs, fecal characteristics, and *Escherichia coli* bacteria count in calves. By examining how various milk replacer formulations

influence these critical indicators of calf health, this research aims to provide valuable insights for optimizing calf-rearing practices and improving overall calf health and productivity.

Materials and methods

Ethical approval

The study was conducted by qualified veterinarians strictly adhering to established research ethics and animal welfare guidelines. The calves were provided with milk in quantities that met their basic nutritional requirements, approximately 10 litres per day. Physiological data were systematically collected, including rectal temperature measurements, respiratory rate assessment through thoracic observation, and heart rate monitoring using a stethoscope placed over the heart region. Fecal characteristics were assessed through the non-invasive collection of samples via digital rectal palpation. Given the non-invasive nature of the procedures and the full compliance with ethical principles such as the 3Rs (Replacement, Reduction, Refinement), ethical approval for using experimental animals was not required for this research.

Study's design

Fifteen Holstein-Frisian bull calves were assigned to three treatment groups (Table 1), with five calves in each group. The calves' body weights ranged from 40 to 50 kg. During a one-week habituation period, the calves were initially between 3 and 4 weeks old. Consequently, they were 4 to 5 weeks old when the study commenced and 8 to 9 weeks old by the end of the study.

Table 1: Ingredients of each milk

Types of Milk	Ingredients
Fresh Milk	Fresh Milk
Commercial Milk	Factory owned
LK Milk	95,960 g commercial milk; 200 g salt, 100 g Betaine, 200 g lysin, 100 g methionine, 300 g calcium butyrate, 50 g curcumin, 1000 g dextrose monohydrate, 10 g coccidiostat, 20 g vitamin A, 20 g vitamin D, 20 g vitamin E, 20 g vitamin K.

The parameters of this study are physiological vital signs, fecal characteristics, and *E.coli* bacterial counts. Physiological vital signs were assessed through heart rate, respiratory rate, and rectal temperature, while fecal characteristics were examined based on colour and consistency. Physiological vital signs and fecal characteristics were assessed every two days in the morning before milking for 30 days. *E.coli* bacterial counts were analyzed on the 1st day, 15th day, and 30th day. Figure 1 illustrates the experimental design.
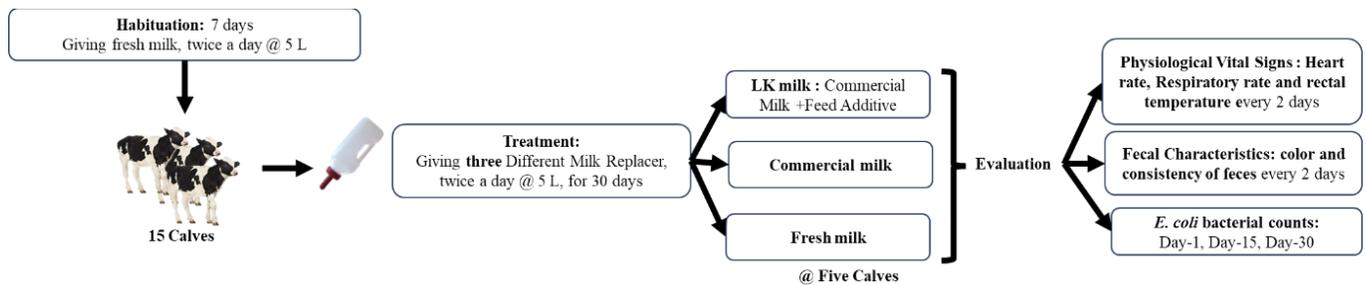

Figure 1: Experimental design.

Physiological vital signs assessment

Physiological vital signs were evaluated by heart rate, respiratory rate, and rectal temperature (5). Heart rate was examined with auscultation of the heart region in one minute. The animals were auscultated, preferably in a standing position and at rest. The respiratory rate was assessed with an inspection of the thorax region in one minute. Rectal temperature was taken with a digital thermometer inserted in the rectum, in contact with the mucosa, until stabilization of the temperature, which was recorded in Celsius degrees (°C). Physiological vital signs were conducted every two days in the morning before feeding milk.

Fecal characteristics evaluation

Fecal characteristics were evaluated based on fecal colour and consistency every two morning days by examining the barn and perineal area. The fecal colour was examined using a modified assessment method (6-7). Fecal consistency was determined based on the modified scoring method (8) (Tables 2 and 3).

***E.coli* bacterial counts**

After feeding milk, approximately three grams of feces were collected using digital rectal palpation with sterile gloves on day 1, day 15, and day 30. Fecal samples were

cultured in eosin methylene blue agar (EMB agar) to analyze *E.coli* bacteria using the pour plate method. One gram of feces was diluted using 9 ml of NaCl and then serially diluted to 10^5. A 1 ml sample was poured into a petri dish, and then 15 mL of EMBA media was poured in and homogenized. The sample was incubated at 37°C for 24 -48 hours in an inverted position. *E. coli* was evaluated with metallic green colonies on EMBA (9). The number of *E.coli* bacteria was calculated between 30-300 colonies in each dilution, and the result was the number of colonies per gram of feces (CFU/g feces).

Table 2: Fecal color scoring assessment

Score	Fecal color
1	Dark or black
2	Brownish
3	Yellowish
4	White scour

Table 3: Fecal consistency scoring assessment

Score	Fecal consistency
1	Normal (firm but not hard)
2	Soft (not form, piles but spreads slightly)
3	Runny (spreads readily)
4	Watery (liquid consistency, splatters)

Statistical analysis

All data are expressed as mean ± SD. Physiological vital signs (parametric data) were analyzed using one-way ANOVA, with Duncan's Multiple Range Test (DMRT) applied for significant differences between treatments at P<0.05. Fecal characteristics (non-parametric data) were evaluated using the Kruskal-Wallis and Mann-Whitney tests if significant differences were observed at P<0.05. The

number of *E. coli* bacteria was transformed using the square root (SQRT) method to ensure significance > 0.05. The transformed data were then analyzed with univariate two-way ANOVA, and DMRT was applied if differences between treatments were significant at P<0.05. Statistical analysis was performed using IBM® SPSS® Statistics version 25.0 (IBM, NY, USA).

Results

Table 4 illustrates the effects of various milk replacers on physiological vital signs, including heart rate, respiratory rate, and rectal temperature. The results indicate that the heart rate for the LK milk group is 74.46±6.99 bpm, with no significant difference (P>0.05) compared to the other groups (77.35±5.94 and 73.76±10.71). The respiratory rate for the LK milk group is similar (P>0.05) to that of the other groups, measured at 41.04±13.72 mpm, 39.95±15.48 mpm and 38.45±8.03 mpm, respectively. Moreover, the rectal temperature for the LK milk group is consistent with that of the other group, recorded at 38.86±0.44 °C (P>0.05), 38.84±0.58 °C, and 38.80±0.4 °C, respectively.

Table 5 outlines the effects of different milk replacers on fecal characteristics. According to the table, all treatments resulted in fecal colours ranging from brownish to yellowish. LK milk exhibited a significantly closer fecal colour to yellowish (P<0.05) compared to commercial milk but was similar (P>0.05) to fresh milk. Furthermore, commercial milk showed no significant difference (P>0.05) in fecal colour compared to fresh milk. The consistency of feces across all treatments did not differ significantly (P>0.05), varying from soft to runny.

Table 6 displays the impact of various milk replacers on E. coli bacterial counts over a 30-day assessment period. The data indicates no significant differences (P>0.05) in E. coli counts among the milk types on any observation days.

Table 4: Effect of Different Milk Replacers on Physiological Vital Signs

Treatment	Physiological Vital Signs (Mean ± SD)		
	Heart rate (bpm)	Respiratory rate (mpm)	Rectal temperature (°C)
LK Milk	74.46±6.99[a]	41.04±13.72[a]	38.86±0.44[a]
Commercial Milk	77.35±5.94[a]	39.95±15.48[a]	38.84±0.58[a]
Fresh Milk	73.76±10.71[a]	38.45±8.03[a]	38.80±0.45[a]

Note: Different superscripts in the same column showed significant differences (P<0.05).

Table 5: Effect of different milk replacers on fecal characteristics

Treatment	Fecal Characteristics (Mean ± SD)	
	Fecal color*	Fecal consistency**
LK Milk	2.45±0.67[a]	2.28±0.65
Commercial Milk	2.23±0.52[b]	2.20±0.58
Fresh Milk	2.39±0.58[ab]	2.10±0.62

Note: * scoring of fecal colour: 1 = dark or black, 2= brownish, 3= yellowish, 4= white scour; **scoring of fecal consistency: 1 = normal (firm but not hard), 2 = soft (does not hold form, piles but spreads slightly), 3 = runny (spreads readily), and 4 = watery (liquid consistency, splatters). Different superscripts in the same column showed significant differences (P<0.05).

Table 6. Effect of different milk replacers on *E. coli* bacterial counts

Treatment	Bacterial count in assessment days (x 10^5 CFU/g feces) (Mean ± SD)		
	D-1	D-15	D-30
LK Milk	1.73±1.02	1.37±0.66	1.42±0.56
Commercial Milk	2.33±1.59	0.65±0.11	1.38±0.57
Fresh Milk	0.80±0.87	1.64±0.81	1.44±0.68

Note: Different superscripts in the same row and column showed significant differences (P<0.05).

Discussion

Monitoring physiological vital signs is essential in health management and disease prevention (10). The physiological monitoring of vital signs includes evaluating heart rate, respiratory rate, and rectal temperature (10,11). The heart rate of the LK milk group is the same (P>0.05) as that of the other groups. Scenzi (11) reported that the heart rate frequency in neonatal calves was 100-150 bpm. Heart rate also decreases from the first period to the fifth week of life (12). Neonatal calves require adaptations of the cardiovascular system over time (12). The cardiovascular system of the neonatal calf has high elasticity and peripheral resistance (12). Neonatal calves also have low systolic volume even though the heart needs to pump blood at a higher systolic rate (12). Therefore, the systolic volume of the neonatal must be increased due to the increased heart rate and the inability to change cardiac output (12). The heart rate of dairy cows is 59.82-72.02 until adulthood (13). In this study, the author analyses that the cardiovascular system of calves aged 4-8 weeks could adapt so that their heart rate values were almost close to those of adult cows.

The respiratory rate of the LK milk group does not differ (P>0.05) from the other group. Silva *et al*. (12) showed that the respiratory rate of the calves 31-60 days old is approximately 41 mpm. The respiratory rate of adult dairy cattle is 26.01−36.69 mpm (13). Respiratory rate is influenced by lung capacity (13). Respiratory function in the first days of life has not yet reached perfect development, so the respiratory rate will be higher (13). Increasing the age of the calf results in complete maturation and homeostasis of the respiratory system so that the respiratory rate will be stable (14). The respiratory rate is affected by external factors such as heat dissipation, which regulates body temperature, and individual factors such as physical exertion and exercise (15). The rectal temperature is also the same between the LK milk and the other groups. This finding is in line with Szenci (11), who showed that the rectal temperature of neonatal calves was between 38.5°C and 39 °C. Similar findings were reported by Silva *et al*. (12), who described that the rectal temperature of calves at 31-60 days old was 38.7 – 38.8 °C. Temperature is affected by metabolic and physiological changes, environmental stresses, animal behaviour, heat production, heat transfer to and loss from their surface, and evaporation (15). Environmental temperature in neonatal calves greatly influences body temperature due to immature metabolism (16).

The authors assume that different milk replacers do not directly impact heart rate, respiratory rate, and rectal temperature. However, they impact changes in growth performance first and then affect physiological vital signs. Kloop *et al.* (17) reported that calves consuming high amounts of milk replacer could increase the feed efficiency, average daily gain, and growth parameters. Furthermore, growth performance can affect the physiological vital signs (8). The physiological vital signs were influenced by intrinsic factors such as physiological condition, sex, age, breed, and reproductive function of the female, in addition to extrinsic factors such as season, environmental temperature, humidity, solar radiation, and physical exertion (14).

Fecal characteristics such as colour and consistency can be used to diagnose diarrhea and detect signs of disease. The colour of feces is affected by the type of feed, bile concentration, and the speed of excretion of feed ingredients and digestion (18). The research shows that fecal colour is typically within the brownish to yellowish range. LK milk resulted in a significantly more yellowish fecal colour than other treatments. The authors suggest this may be due to a unique ingredient in LK milk, precisely 50 g of curcumin per litre of milk. Curcumin, a yellow pigment derived from *Curcuma longa*, likely affects the fecal colour. The fat content also influences fecal colour in calves in milk replacers. Research by Kumar *et al.* (19) shows that high-fat milk replacers may cause darker, more formed feces due to slower digestion and longer gastrointestinal transit times. These dietary factors can also affect nutrient absorption and gut fermentation, further influencing fecal characteristics. Feces with abnormal colours, such as red and black, indicate bleeding in the digestive tract (7). Light-green or yellowish fecal colour combined with watery diarrhea is caused by bacterial infections such as salmonella. Diarrhea with yellow-to-white fecal colour sings to *E.coli* bacterial infection (20). Singh *et al.* (21) reported *E.coli* bacterial infection due to diarrhea with watery white or yellowish feces.

The type of milk did not affect fecal consistency. The fecal consistency in the LK milk group (2.28±0.65) was not significantly different from the other treatments. This finding is consistent with the report by Amanulloh *et al.* (22), which indicates that the fecal consistency in calves averages 2.12 (on a scale of 1-4). The water content and the length of the food in the animal's body determine the consistency of feces. Firmer feces are caused by restricted water or protein intake and severe dehydration. Fecal consistency describes the

incidence of diarrhea in calves, especially a score of 4 or watery feces (liquid consistency, splatters) (23). Noninfectious causes of diarrhea are nutritional, immunological, and environmental. Infectious agents due to diarrhea are rotavirus, BCoV, BVDV, *E.coli, Salmonella enteritis, Cryptosporidium* spp, and *Eimeria* spp. (24). *S. enterica, E. coli,* and *C. perfringens* are major enteric pathogens that cause calf diarrhea (25). Trindade-Goncalves *et al.* (26) stated that diarrhea caused by *E.coli* causes the feces to become watery, followed by a paste texture.

E. coli are ubiquitous gastrointestinal tract bacteria and one of the most important zoonotic pathogens. *E. Coli* is also a common cause of diarrhea and septicemia in calves (9). All treatments had a positive impact by not causing diarrhea due to *E. coli* because the number of *E. coli* was still within the non-infective dose in one month. The concentration/dose and duration of *E.coli* infection determine the appearance of clinical symptoms in calves (9). Besser *et al.* (27) stated that no calves were infected when challenged with *E.coli* bacteria at a dose of 10^8 CFU/gram. Cray and Moon (28) also found that *E. coli* O157:H7 at a dose of 10^7 CFU/gram was still insufficient to infect all adult cows, and a dose of 10^4 CFU/gram had no effect on four adult cows. Research by Esfandiari *et al.* (7) showed that the *E.coli* K-99 (ETEC) challenge test at a 5×10^{10} CFU/gram dose produced significant clinical symptoms of diarrhea.

Milk is a source of feed for calves and a substrate for microbiome metabolites in the digestive tract. The balance of the microbiome in the digestive tract plays a vital role in the animal's digestion, absorption, and utilization. Dietary changes can significantly impact the microbiome's composition in terms of the number and type of flora. An imbalance between microflora and pathogenic bacteria in the digestive tract causes pathogenic bacteria to produce toxins. Toxins reduce the ability to digest and absorb, resulting in diarrhea (29). He *et al.* (30) stated that pathogenic *E. coli* infection could induce diarrhea and imbalance the microbiome in the gastrointestinal tract. The author believes that different types of milk have no significant effect on the balance of microflora during 30 days of administration. This can be seen from the number of *E. coli* bacteria in the feces, which is still within the average concentration.

Calves can become infected with pathogenic *E. coli* by ingesting contaminated calf bedding and buckets, perineal skin, calves experiencing diarrhea in crowded calving areas, and dirty calf pens (20). Besser *et al.* (27) described the factors that affect the infectivity of *E. coli* as strain differences, dietary effects, and seasonal effects. The pathogenicity of *E.coli* is also affected by the strain, exposure time, and the host's immune system (20). The Cattle age also affects the *E.coli* infection. The research from Singh *et al.* (21) reported that at 1-3 days and 3-8 weeks, calves are easily exposed and infected with *E. coli*. Adult cattle have a fully developed forestomach compartment so that the development of *E. coli* can be inhibited through low pH and high VFA concentrations (28). Chuan-qi *et al.* (29) stated that acidic conditions inhibit pathogenic bacteria and increase *Lactobacillus* bacteria.

Conclusion

Differences in the type of milk replacer given to calves do not affect physiological vital signs, fecal characteristics, or the number of *E. coli* bacteria in feces. Further study is needed to evaluate the effects of milk replacers against specific strains of *E. coli* and other infectious agents.

Acknowledgment

Conflict of interest

The author declares that they have no competing interests.

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تأثير بدائل الحليب المختلفة على العلامات الحيوية الفسيولوجية وصفات البراز وعدد بكتيريا الإشريكية القولونية في العجول

إندانغ يوني سيتيواتي¹، فيصل أمري ساتريو²، سبتياني سبتياني²، ساراساتي ويندريا²، و محمد فتح وايتنا¹

¹قسم الإنتاج الحيواني، كلية الثروة الحيوانية، ²قسم العلوم الطبية الحيوية، كلية الطب، جامعة بادجادجاران، باندونج، إندونيسيا

الخلاصة

تهدف هذه الدراسة إلى تحديد العلامات الحيوية الفسيولوجية وخصائص البراز وعدد بكتيريا الإشريكية القولونية في العجول التي تعطى أنواعا مختلفة من الحليب. تم استخدام ما مجموعه ١٥ عجلا ذكرا يبلغ وزنها حوالي ٤٠-٥٠ كغم في هذا البحث. تم تقسيم العجول إلى ثلاث مجموعات علاجية: LK أو الحليب التجاري أو الطازج. تم تقييم العلامات الحيوية الفسيولوجية وخصائص البراز كل يومين في الصباح لمدة ٣٠ يوما. تم تقييم العلامات الحيوية الفسيولوجية بناء على معدل ضربات القلب ومعدل التنفس ودرجة حرارة المستقيم. تم أخذ عينات لعدد بكتيريا الإشريكية القولونية في الأيام ١ و ١٥ و ٣٠. تم استزراع عينات البراز على وسط أكار EMB باستخدام طريقة صفيحة الصب. أظهرت النتائج أن مجموعة حليب LK كان لها نفس معدل ضربات القلب وتردد التنفس ودرجة الحرارة مثل المجموعات الأخرى. نتج عن جميع العلاجات ألوان البراز التي تتراوح من البني إلى المصفر. أظهر حليب LK لونا برازيا أقرب بكثير إلى اللون الأصفر من الحليب التجاري ولكنه كان أكثر تشابها مع الحليب الطازج، الذي يميل إلى اللون البني. كان تناسق البراز متشابها في جميع مجموعات العلاج، بدءا من اللينة إلى السائلة، مع عدم وجود فروق ذات دلالة إحصائية. بالإضافة إلى ذلك، لم يكن لنوع الحليب تأثير كبير على عدد بكتيريا الإشريكية القولونية التي لوحظت طوال فترة الدراسة. وبالتالي، فإن الاختلافات في نوع الحليب المعطى للعجول لا يؤثر على العلامات الحيوية الفسيولوجية وخصائص البراز وعدد بكتيريا الإشريكية القولونية في البراز.

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