SPECIAL INTEREST SECTION

Beneficial microbes and their combinations improved the growth and health performance of female Holstein calves (*Bos taurus*)

RANDY BERGOLA GAQUIT[1], RUDY CASTRO FLORES[2*], PEDRO VICTORY DUQUE[1] and JONATHAN MANZANO SALAS[2]

[1] Poaka Farm, New Zealand Faculty of Animal Science, College of Agriculture

[2] Bataan Peninsula State University- Abucay Campus, Philippines

Correspondence: rcflores@bpsu.edu.ph

Abstract

This study was conducted to evaluate the growth and health performance of Holstein Friesian calves (*Bos taurus*) supplemented with beneficial microbes and their combinations at varying levels for 30 days from 1st September 2021 to 1st October 2021 in Poaka Farm, Tokoroa, New Zealand. A total of 48 female calves were randomly distributed in 8 treatments, having 3 replications with 2 calves per replication, following a factorial experiment in a completely randomised design.

Factor A was the types of beneficial microbes (BM): BM_1- *Lactobacillus subtilis,* BM_2- *Bifidobacterium animalis,* BM_3- Lactic Acid Bacteria Serum, and BM_4- *Lactobacillus* + *Bifidus* + LABS.

Factor B was the levels of supplementation (LS): LS_1- 10ml per calf/day and LS_2- 20ml per calf/day.

The parameters were: feed consumption, milk consumption, weight gained, average daily gain (ADG), feed conversion ratio (FCR), feed and milk cost, income, morbidity, survival, faecal colour, faecal odour, faecal consistency, degree of scouring and general appearance scores.

Results of the study revealed a highly significant difference among means on the average feed consumption which indicate a positive effect caused by the types of beneficial microbes and the levels of supplementation. The significant improvement in feed consumption was the beneficial effect of the *Lactobacillus subtilis, Bifidobacterium animalis*, and Lactic Acid Bacteria Serum due to enhanced intestinal health and an increased digestive capacity of the experimental calves.

While the remaining parameters assessed were not significantly affected by the types of beneficial microbes and levels of supplementation. The diverse types of beneficial microbes and their combinations at varying levels can be used as supplement for the calves to enhance intestinal health is important for increasing their digestive capacity in order that they will consume more feeds and therefore increase their growth rate.

Introduction

Importance of the study

Between June 2011 and June 2020, 4.19 million dairy calves on average were born in New Zealand.[1] Approximately ¼ are kept as replacement heifers.[2] New Zealand's dairy system is primarily pasture-based. Calves are raised on pasture grass from infancy; and thus potential risk factors for mortality and morbidity. The management of calves in the first 3 weeks of life is critical for their health and longevity.[3,4,5] During this time neonatal calves are particularly susceptible to infectious gastrointestinal and respiratory diseases.[3,6] Diarrhoea can have both infectious and non-infectious aetiologies and co-infection is common in scouring calves.[7,8,9]

Probiotic microorganisms benefit from a natural image and can expect a promising future in animal nutrition. Controlled research studies demonstrate that they can positively balance gastrointestinal microbiota and improve animal production and health.[10] Probiotics may play a role in suppressing the injurious effect derived from the instability of microflora colonisation, encouraging immunisation and inhibiting epithelial and mucosal adherence and epithelial invasion by harmful pathogens such as entero-toxigenic *Escherichia coli*.[11] The combination of probiotics and phytobiotics as a feed additive to the diet of dairy calves during rumen fermentation and biochemical blood indices, including the inclusion of phytobiotic with rosmarinic acid as the main bioactive components or probiotics does not affect growth performance and physiology indices.[12] But probiotics may be alternative to the antibiotics commonly used as growth promoters in calves.[13] Probiotic administration before weaning could improve calf health and decrease mortality of 0.04% and medication.[14]

Lactic Acid Bacteria Serum (LABS) is an anaerobic microorganism that decomposes sugar without oxygen which are separated and cultured with rice-washed water and milk.[15] These should maintain their viability during animal feed processing and storage.[16] Beneficial microbes or probiotic feed additives are attracting increased attention as a cost-effective alternative to controlling animal disease.[17]

This study attempted to improve the growth and health performance of new-born calves by using *Lactobacillus subtilis, Bifidobacterium animalis* and homemade Lactic Acid Bacteria Serum (LABS) and their combinations as a supplement to be drenched once or twice daily. The general objective of this study was to evaluate the growth and health performance of the calves supplemented with different beneficial microbes and their combinations at varying levels. Specifically, this study aimed to assess their growth and health performance in terms of feed consumption, milk consumption, gain in body weight, average daily gain, feed conversion ratio, feed and milk cost per kilogram (kg) of gain in weight, income over feed, milk, beneficial microbes and calf cost, morbidity rate, survival rate, faecal colour score, faecal odour score, degree of scouring score, faecal consistency score and general appearance score.

Materials and Methods

Animals

This study used a total of 48 Holstein Friesian female calves raised in Poaka Farm, Tokoroa, New Zealand that were selected from the 600 newborn calves at the dairy farm. Only healthy female calves that were five days old with an average of 29.41 kg bodyweight at the start of the experiment were selected to avoid discrepancies and to minimise bias. Those chosen were randomly distributed in a factorial experiment following a completely randomised design (CRD).

The experimental house was prepared by thorough cleaning and disinfection one week before the arrival of the calves. The shed had sufficient ventilation to ensure a regular circulation of clean air through it to ensure the calves remained warm and dry. Each calf was provided with 2 square meters (2m^2 to allow the calves to move around freely, explore and play. An all-in all-out method which is the practice of keeping animals together in groups, avoiding animals from different groups to mix during their stay on the farm, which are closely matched by age, weight, production stage and condition. The group is moved into a phase of production together, such as into an empty nursery and is moved out of that phase as a group according to a production schedule. When a group moves forward, the facility is completely emptied. This was done to minimise scours and animal health issues. Bedding was changed every 10 days to ensure that calves remained dry. They had access to fresh water and feed and their environmental temperature range was between 15 to 25°C as it was Spring time in New Zealand at the time of the experiment.

A strict biosecurity measure was observed during the experimental period to ensure the calves' health protection. The entry of an unauthorised personnel in the area was prohibited which helped to reduce spreading dust and dirt inside the pens. Cleanliness was maintained in the experimental area by regular disinfection and a clean water supply. Removal of manure and replacement of litter materials was performed weekly to prevent the accumulation of ammonia and to prevent the entry of pathogens that could cause diseases to the calves.

The calves were placed in pens according to the proposed treatments after taking their initial bodyweight. Body-weights were performed every 7 days to monitor their growth and development. Their final bodyweight was taken when they reached 35 days old.

Preparation of supplements

To prepare the Lactic Acid Bacteria Serum *(LABS)*, the following materials are needed:

- 1kg uncooked rice, molasses or brown sugar
- 1L fresh or pasteurised milk

- unchlorinated clean water
- plastic or glass clear jar or container
- a plastic basin
- clean cloth as a strainer
- digital weighing scale
- funnel
- paper
- rubber band

The steps to follow are:

1. Make 'rice washing' by adding 700ml of clean water to 1kg of uncooked rice.
2. Stir, swirl and crush by hand until milky-coloured water was attained from the mixture.
3. Obtain the rice washing by filtering the mixture using a clean cloth.
4. Place 500ml rice washing in a clear jar and cover with paper and a rubber band.
5. Leave the jar in a cool dark place for 5 to 7 days.
6. When the bran has risen, it will smell a little sour and form 3 layers, this has indicated the rice washing is infected with various microbes.
7. 100ml of clear water was taken by straining the mixture again.
8. 1L of fresh milk was added to 100ml clear water after straining to obtain a 1:10 water and milk ratio.
9. The clear jar was covered with paper again and left for 5 to 7 days in a cool dark place.
10. Curds appeared after 7 days (made of carbohydrates, protein and fat) or the white part and yellow liquid or whey, enriched with lactic acid bacteria seen from the fermentation of the milk.
11. The whey was extracted by pouring it through a strainer to create serum.
12. Molasses/brown sugar in equal amounts were weighed and added to the collected whey (serum)
    – This acts as a stabilising agent for the bacteria, keeping the bacteria alive in a stable dormant stage at room temperature.
13. The produced LABS was preserved at room temperature by placing it in a clean container with a loose cap to avoid air pressure increase and labelled accordingly.[15]

Experimental procedure

The experimental calves were fed with the required amount of 1kg per calf per day starter feeds for 30 days, followed by restricting their food the restricted system of feeding. Fresh drinking water was always provided in buckets and available. A commercially available preparation of *Lactobacillus subtilis* containing $10x10^8$ microbes per L was used as a source of beneficial microbes. 1ml of *Lacto bacillus subtilis* was mixed with 1L of water, containing an estimated 10^6 microbes, before drenching to each calf assigned for the treatment. Another source of beneficial microbes available commercially is *Bifidobacterium animalis.* This preparation contains $10x10^9$ microbes per kg. One gram of *Bifidobacterium animalis* was mixed with 1L of drinking water to obtain an estimated 10^8 microbes before drenching it to each calf assigned for the treatment.

Factor A beneficial microbes (BM)

- BM_1- *Lactobacillus subtilis,*
- BM_2- *Bifidobacterium animalis*
    - BM_3- Lactic Acid Bacteria Serum (LABS)
    - BM_4- *Lactobacillus subtilis* + *Bifidobacterium animalis* + LABS

Factor B varying levels of supplementation (LS):

- LS_1- 10ml per calf/day
- LS_2- 20ml per calf/day

The experimental treatments were as follows:

- BM_1LS_1- 10ml *Lactobacillus subtilis* per calf/day
- BM_1LS_2- 20ml *Lactobacillus subtilis* per calf/day
- BM_2LS_1- 10ml *Bifidobacterium animalis* per calf/day
- BM_2LS_2- 20ml *Bifidobacterium animalis* per calf/day
- BM_3LS_1- 10ml LABS per calf/day
- BM_3LS_2- 20ml LABS per calf/day
- BM_4LS_1-10ml *Lactobacillus subtilis* + *Bifidobacterium animalis* + LABS per calf/day
- BM_4LS_2-20ml *Lactobacillus subtilis* + *Bifidobacterium animalis* + LABS per calf/day

Drenching of 10ml and 20ml of the prepared *Lactobacillus subtilis, Bifidobacterium animalis,* and LABS was performed every morning for 30 days. A plastic drencher was used to administer the preparation to avoid spoilage. Each calf was correctly restrained to avoid injury to both the researcher and calves. It was performed in a gentle manner of handling by approaching the head from the side (not the front), running the hand from the neck under the ear and along the jawbone, then cup the jaw in one hand from the animals head in case it jerks up, making sure that personal protective equipment were worn and safe work standards followed.

The following data was gathered and analysed using the analysis of variance for a CRD: average initial weight (AIW), average feed consumption (AFC), average milk consumption (AMC), average gain in weight (AGW), average daily gain (ADG), feed conversion ratio (FCR), average feed and milk cost per kilogram of calf produced (FCKCP), average income over feed, milk, beneficial microbes and calf cost (IOFMBC), average morbidity rate, average survival rate. Meanwhile, to determine the influence of different beneficial microbes at varying levels on the health performance of the experimental calves, faecal samples were collected and evaluated daily using a manure scoring guide.[22,23]

Manure score guide

All the data gathered was analysed following the analysis of variance (ANOVA) for a factorial experiment in a Completely Randomised Design (CRD). Comparison among means was obtained using the Least Significant Difference (LSD).

Texture	Score
Manure with normal (firm to soft) consistency; brown to light brown colour; normal odour	1
Manure with soft to loose consistency; yellow, brown, or green; mucus; slight odour	2
Manure with Loose to Watery consistency; yellow or green colour; mucus; strong odour	3
Manure with Watery consistency; yellow, green, or clear colour; mucus; slight blood; strong odour	4
Manure with Watery consistency; clear colour, mucus; bloody	5

Results

Growth performance parameters

The parameters on the growth performance of the calves included the average feed consumption, milk consumption, weight gain, ADG, feed conversion ratio, feed and milk cost to produce a kg weight gain, income over feed, milk and calf cost.

The results of the experiment revealed the following:

1. A highly significant difference in the average feed consumption (Figure 1) of the experimental calves was obtained (P<0.05). This indicates that the feed consumption was highly affected by the types of beneficial microbes and the level of supplementation due to their ability to enhance intestinal health resulting in increased digestive capacity. The average feed consumption of the experimental calves presented in Figure 1 shows that those given with *Lactobacillus* + *Bifidus* + LABS (BM_4) supplement consumed the highest number of feeds with an average of 1.80kg/day followed by *Bifidobacterium animalis* (BM_2) and *Lactobacillus subtilis* (BM_1) with 1.79kg and 1.56kg, respectively. The lowest number of feeds consumed was observed in calves assigned in BM_3 (Lactic Acid Bacteria Serum or LABS) with a mean value of 1.52kg. Levels of supplementation, as drenched, showed that the calves in LS_2 (20ml per calf/day) had a higher average feed consumption of 1.72kg compared to the 1.62kg average feed consumption of calves in LS_1 (10ml per calf/day). For the interaction between the types of beneficial microbes and levels of supplementation (BM x LS), as reflected in Figure 1, calves supplemented with combinations of *Lactobacillus subtilis, Bifidobacterium animalis,* and LABS (BM_4LS_2-20mL L+B+LABS per calf/day) with a mean value of 1.92kg obtained the highest mean on average feed consumption, followed by BM_2LS_1 (10mL *Bifidobacterium animalis* per calf/day) with 1.86kg, while calves in BM_1LS_2 (20ml *Lactobacillus subtilis* per calf/day), BM_2LS_2 (20mL *Bifido bacterium animalis* per calf/day), BM_4LS_1 (10ml L+B+LABS per calf/day), BM_3LS_1 (10mL LABS per calf/day), and BM_3LS_2 (20ml LABS per calf/day) consumed an average of 1.75kg, 1.71kg, 1.69kg, 1.55kg, and 1.49kg feeds, respectively. The lowest mean was observed in BM_1LS_1 (10mL *Lactobacillus subtilis* per calf/day) with 1.38kg average feed consumption.

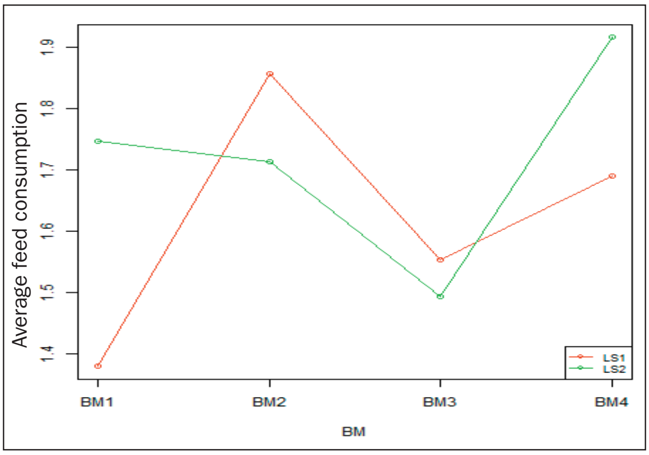

Figure 1. Average feed consumption per kg.

2. The analysis of variance revealed no significant difference among treatment means among the different types of beneficial microbes, including their combinations and the varying levels of supplementation on the average milk consumption (Figure 2), average gain in weight (Figure 3), average daily gain (Figure 4), feed conversion ratio (Figure 5), feed and milk cost per kg of gain in weight (Figure 6), income over feed, milk, beneficial microbes and calf costs (Figure 7).

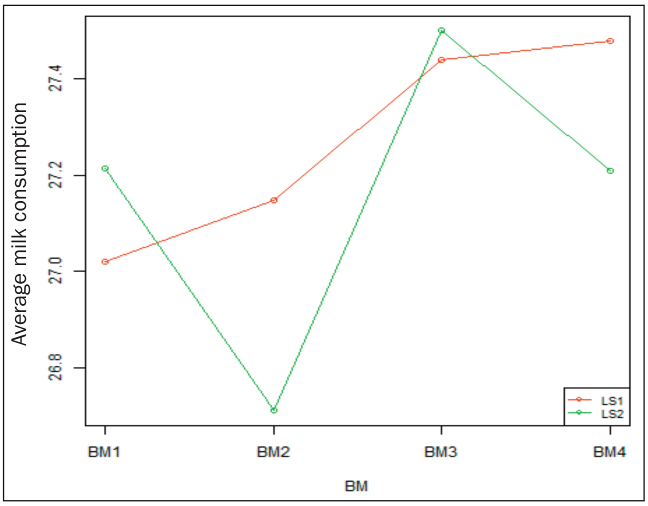

Figure 2. Average milk consumption per L.

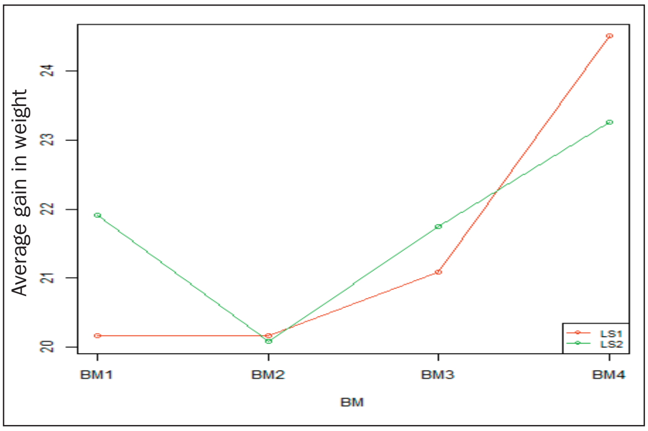

Figure 3. Average gain in weight per kg.

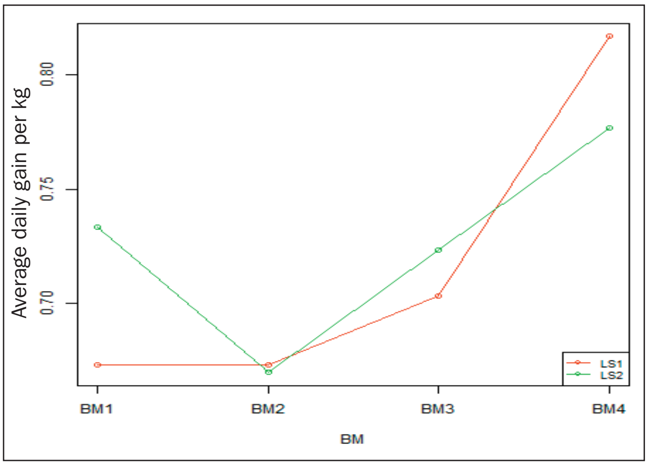

Figure 4. Average daily gain per kg.

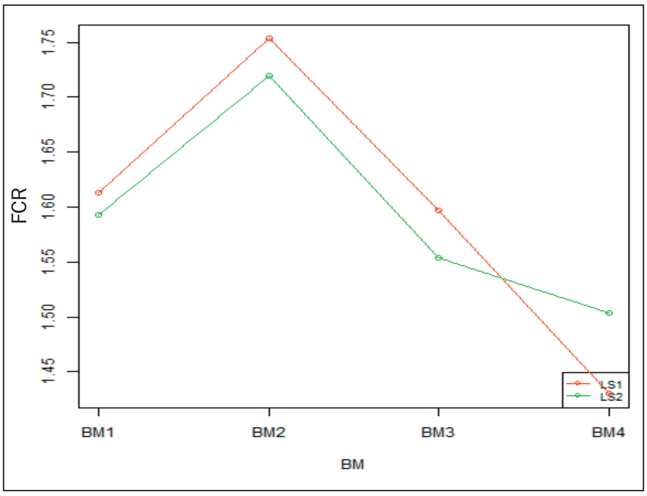

Figure 5. Feed conversion ratio.

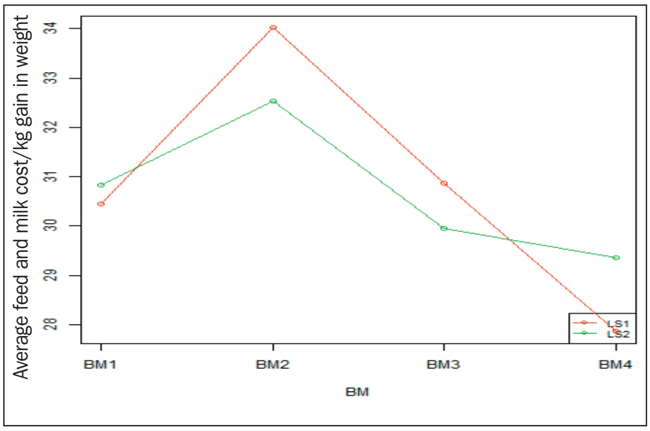

Figure 6. Average feed and milk cost/kg gain in weight, NZ$

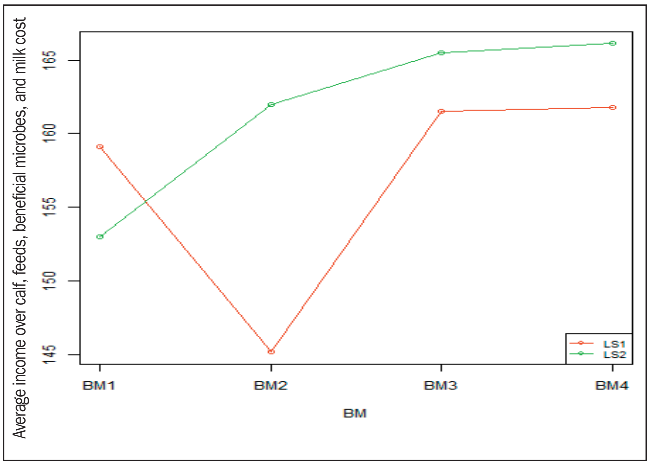

Figure 7. Average income over calf, feeds, beneficial microbes, and milk cost in NZ$.

Health performance

Statistically, there were no significant differences among means on the morbidity rate (Figure 8), survival rate (Figure 9), faecal colour (Figure 10), faecal odour (Figure 11), faecal consistency (Figure 12), degree of scouring (Figure 13), and average general appearance (Figure 14) scores of the calves used in this study (P>0.05).

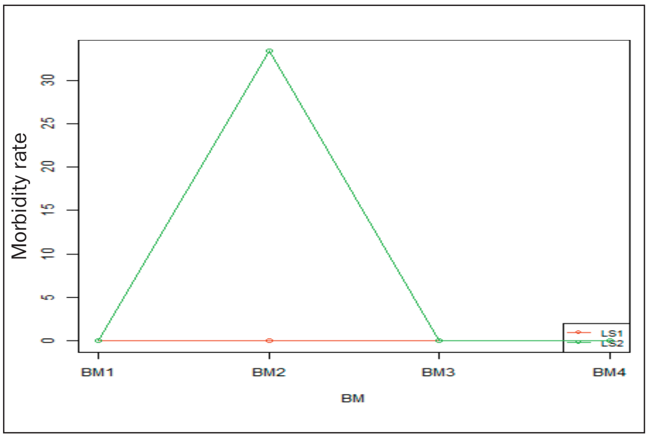

Figure 8. Morbidity rate.

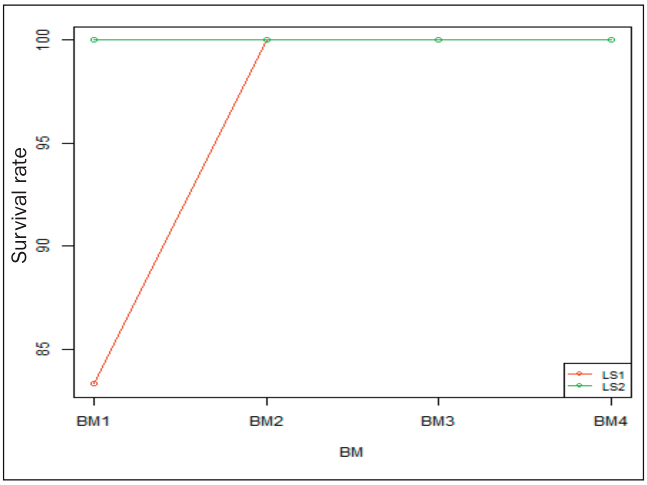

Figure 9. Average % survival rate.

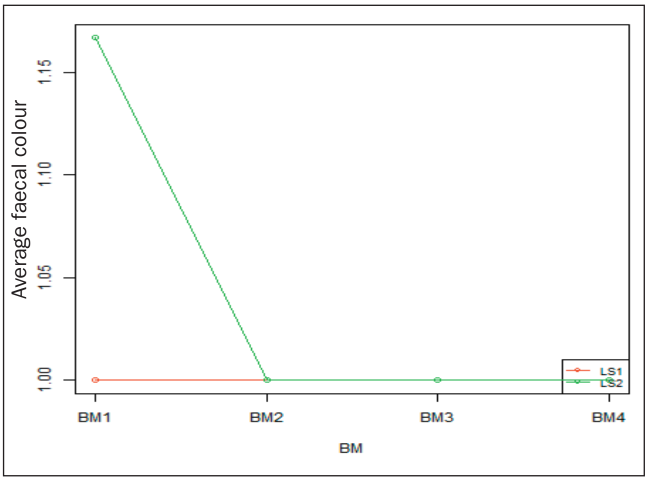

Figure 10. Average faecal colour score.

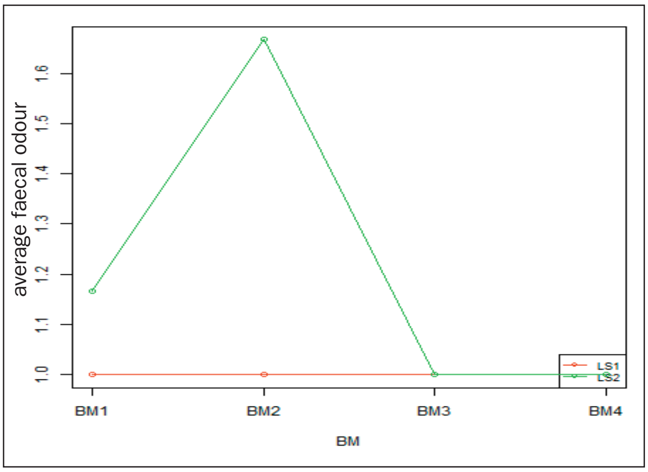

Figure 11. Average faecal odour score.

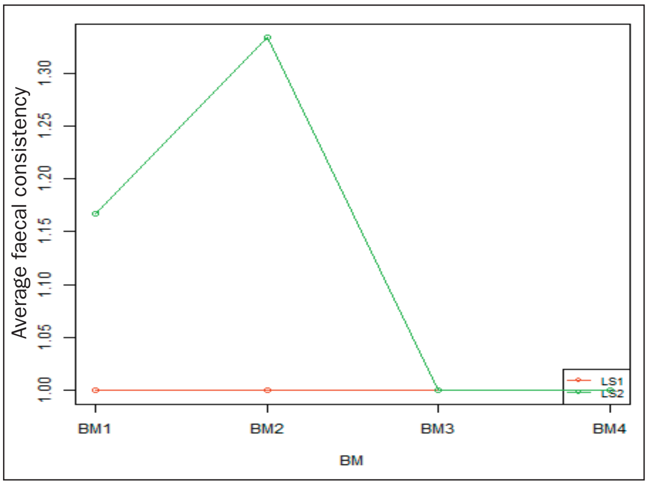

Figure 12. Average average faecal consistency score.

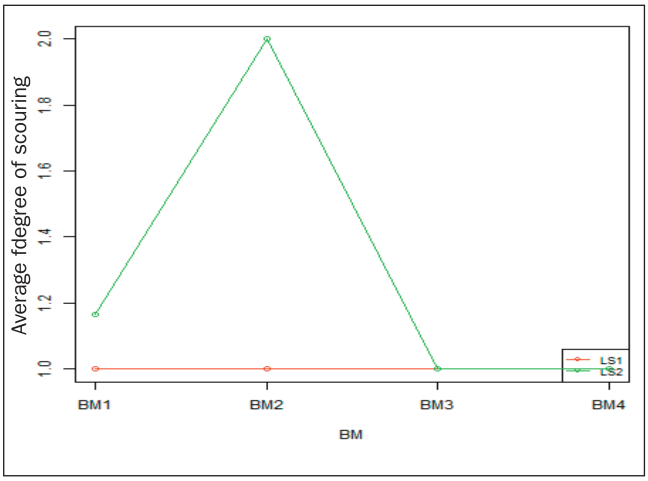

Figure 13. Average degree of scouring score.

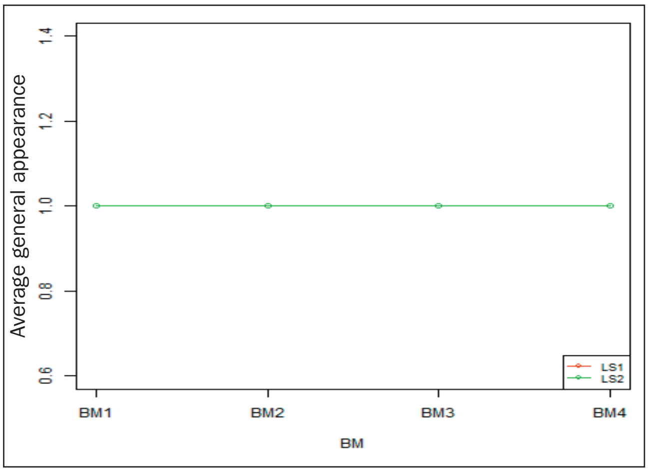

Figure 14. Average general appearance score.

Discussion

Growth performance

There is a highly significant difference among treatment means on the average feed consumption of the experimental calves used in this study (P<0.01). This indicated that the types of beneficial microbes including their combination and the varying levels of supplementation significantly affected the average feed consumption. Comparison among means on the type of beneficial microbes revealed that BM_2 (*Bifidobacterium animalis*) and BM_4 (*Lactobacillus subtilis + Bifidobacterium animalis* + LABS) were comparable with each other but significantly higher over BM_1 (*Lactobacillus subtilis*) and BM_3 (LABS) at (*P<0.01*). This means that the average feed consumption was significantly affected by the types of beneficial microbes used as supplements in this experiment. This significant improvement in feed consumption supports what was mentioned in a review that lactic acid bacteria (LAB) strains, species belonging to the genera *Lactobacillus*, *Bifidobacterium*, and *Enterococcus*, are considered beneficial due to their ability to enhance intestinal health by stimulating the development of a healthy microbiota (predominated by beneficial bacteria), preventing enteric pathogens from colonising the intestine, increasing digestive capacity, lowering the pH and improving mucosal immunity.[18] Mean comparison on the levels of supplementation shows that LS_1 (10ml per calf/day) is significantly higher than LS_2 (20ml per calf/day). This means that the average feed consumption of the calves was significantly affected by the levels of supplementation. These findings coincide with what was noted[19,20] as cited that the use of direct-fed microbes (DFM) in young calves causes the rapid establishment to adapt to solid feed by stabilising the rumen and intestinal microbes,[18] resulting in increased digestive capacity.[18]

Meanwhile, the milk consumption, gain in weight, ADG, FCR, feed and milk cost per kilogram gain in weight, income over feed, milk, beneficial microbes and calf cost revealed no significant difference among treatment means on the different types of beneficial microbes and the levels of supplementation based on the analysis of variance (P>0.05). This indicates that the types of beneficial microbes including their combination and the varying levels of supplementation had not significantly affected these parameters.

Health performance

The analysis of variance revealed no significant difference among treatment means on the morbidity rate, survival rate, faecal colour score, faecal odour score, faecal consistency score, degree of scouring score and the general appearance score of the calves (P>0.05). These indicate that the different beneficial microbes and their combinations at varying levels have not significantly affected the health parameters evaluated in this study.

This study proved that the different beneficial microbes could be used as supplements due to improved feed consumption. The significant improvement in feed consumption was due to the beneficial effects of the *Lactobacillus subtilis, Bifidobacterium animalis* and Lactic Acid Bacteria Serum and their ability to enhance intestinal health, therefore increasing their digestive capacity.

Since no significant differences existed among means on all other parameters evaluated in this study, the use of either *Lactobacillus subtilis, Bifidobacterium animalis*, Lactic Acid Bacteria Serum (LABS) or their combinations at 10 ml or 20 mL per calf/day was found to be beneficial.

The distinct types of beneficial microbes and their combinations at varying levels can be used as supplement to enhance intestinal health, which is important for increasing the digestive capacity of young calves in order that they will consume more feeds and, therefore, increase their growth rate.

A follow-up study on using these beneficial microbes at varying levels for a longer period to further evaluate their benefits on the growth and health of young animals is also recommended.

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