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## Effects of sodium butyrate and active *Bacillus amyloliquefaciens* supplemented to pasteurized waste milk on growth performance and health condition of Holstein dairy calves

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### ABSTRACT

The objectives of the present study were to examine the impact of feeding both probiotics and sodium butyrate on calf performance and the economic implication of each treatment. A completely randomized design was used to investigate body weight (BW) gain, feed conversion efficiency and health conditions of Holstein dairy calves fed either pasteurized waste milk (PWM;  $n = 9$ ) or a non-medicated milk replacer containing sodium butyrate and active probiotic *Bacillus amyloliquefaciens* (NMR;  $n = 9$ ) from birth to 60 days of age. Numerically, calves fed PWM consumed more starter feed between days 16 and 45 than calves fed NMR but the difference became smaller by 60 days. Birth weights and colostrum IgG and serum total protein concentrations did not differ ( $p > 0.05$ ) between the PWM and NMR calves. Calves receiving PWM had slightly greater BW at days 30 and 45, but were similar to that of calves receiving NMR at day 60. No differences were observed between PWM and NMR-calves for BW gains, flank height, hip width and health conditions ( $p < 0.05$ ). Calves fed NMR had more watery feces but less frequent bouts of coughing than PWM-fed calves. Feed cost was higher ( $p < 0.001$ ) for PWM-fed calves than NMR-fed calves during the experimental period. Dairy calves receiving NMR fortified with sodium butyrate and *Bacillus* probiotic could perform as similar as calves receiving PWM, and they had similar economic efficiency during the 60-d study period.

### KEYWORDS

Calf; milk replacer; growth performance; probiotics; sodium butyrate

## Introduction

Increasing interest is paid on dairy calf growth through enhancing early life nutrition, based on increased liquid feeding to enhance calf survival and growth.<sup>1–3</sup> Such practice would also provide better support to mammary gland development and subsequently improve future milk production.<sup>1</sup> In general, liquid feeds include commercial milk replacers, whole milk, pasteurized waste milk and non-saleable milk.<sup>2</sup>

Waste milk is defined as non-saleable milk from transition cows, or milk from cows treated with antibiotics. Waste milk is generally not suitable for human

consumption, but it contains essential nutrients that can support calf growth. Godden et al.<sup>2</sup> showed that about 22 to 62 kg of waste milk is discarded per cow each year in USA, resulting in large economic loss to the dairy industry. However, feeding raw non-saleable waste milk may pose risks of transmission of infectious pathogens, such as *Mycobacterium avium*, *M. bovis*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella*, *Mycoplasma*, and *Campylobacter*. Godden et al.<sup>2</sup> showed that pasteurization is an effective method to destroy common pathogenic bacterial species in waste milk.

Non-medicated milk replacer is widely used to replace whole milk for dairy calf feeding as it is less costly<sup>3</sup> and easily stored in powder form.<sup>4</sup> Moreover, milk replacer can be manufactured with a variety of ingredients and at different levels of nutrients together with additives, such as antibiotics, coccidiostats, probiotics and ionophores to improve calf growth. Elghandour et al.,<sup>5</sup> in their review paper, reported less incidence and duration of diarrhea in calves consuming probiotics (e.g., *Saccharomyces cerevisiae*, *Bacillus amyloliquefaciens* and *S. boulardii*), which may reduce mortality and morbidity of neonatal calf as a result of a reduction of pathogenic bacteria such as *E. coli* and *Salmonella*.<sup>6</sup> Supplementation of probiotics may accelerate the establishment of ruminal and intestinal microorganisms and reduce the establishment of enteropathogens, which often cause diarrhea.<sup>5</sup> Kowalski et al.<sup>7</sup> observed that the inclusion of probiotics (*B. licheniformis* and *B. subtilis*) in milk replacer and starter feeds increased starter feed intake and daily gain without affecting milk replacer intake, health status and a faecal score of calves until the age of 10 weeks. Conversely, Riddell et al.<sup>8</sup> did not observe an impact of a *Bacillus*-based probiotic on growth performance or scouring in pre-ruminant calves. Nonetheless, they noted a marked increase in blood beta-hydroxybutyrate (BHB) concentration as the probiotic supplementation progressed. The elevated BHB concentrations indicated that probiotic-supplemented calves likely had greater ruminal development as butyric acid, the precursor of BHB, is a major volatile fatty acid produced during ruminal fermentation.

Supplementation of butyric acid has been shown to have a positive impact on growth performance by enhancing proliferation, differentiation and function of gut tissues in both healthy and sick animals.<sup>9</sup> Sodium butyrate is often supplemented instead of butyric acid because the salt is more stable and less odorous.<sup>9</sup> Supplementation of sodium butyrate in milk replacer improves feed conversion efficiency and growth rates of neonatal calves by stimulating rumen (e.g., papillae growth) and small intestinal development.<sup>9,10</sup> Moreover, the sodium butyrate supplementation potentially improves the efficacy of gastrointestinal tract digestion as indicated by enhanced production of digestive enzymes as well as increased absorptive capacities in the upper small intestine.<sup>9</sup> Gorka et al.<sup>10</sup> observed that sodium butyrate supplemented in milk replacer tended to increase average daily gain of calves but was associated with greater incidence of scours. We hypothesize that supplementation of probiotics and sodium butyrate in the

milk replacer would result in higher daily gains with a lower incidence of scours. To the best of our knowledge, no study has investigated into the effects of both *Bacillus*-based probiotic and sodium butyrate included together in a milk replacer and compare it with feeding whole milk. Therefore, the objective of this study was to compare feed intake, body weight gain and average daily gain, feed conversion efficiency, economic efficiency, and health conditions of Holstein calves fed either pasteurized waste milk or non-medicated milk replacer containing sodium butyrate and active *B. amyloliquefaciens* until 60 days of age.

## Materials and methods

This study was conducted on a commercial dairy farm in Pavilion Arteaga (Aguascalientes, Mexico). All experimental procedures were performed in accordance with the ethical standards of the official Mexican standard of animal care (NOM-051-ZOO-1995) and the 1964 Helsinki declaration and its later amendments for comparable ethical standards.

### Calves, management and feeding

Eighteen clinically healthy Holstein female calves with an initial BW of  $41 \pm 3.7$  kg (mean  $\pm$  standard deviation), born without congenital malformations, were separated from their mothers within 1–2 h from birth. Calves were identified with an ear tag (Inteltec<sup>®</sup>, Inteltec S.A. de C.V., Mexico) and fed colostrum containing 70–100 mg/mL immunoglobulin G (IgG, analyzed according to Biemann et al.<sup>11</sup>) within the first 2 h of birth at the rate of 10% of their BW. Colostrum was pooled from all cows and then fed to calves individually using a bottle. Calves were offered 2 L of colostrum every 12 h for three days as a sole feed and then housed in individual metal pens (1.2 m  $\times$  1.5 m) bedded with sawdust for an experimental period of 60 days. Calves were randomly assigned to one of two dietary treatments: pasteurized waste milk (PWM;  $n = 9$ ) or non-medicated milk replacer containing sodium butyrate and active probiotic *B. amyloliquefaciens* (NMR;  $n = 9$ ). The PWM was raw milk containing (g/kg): fat (32.3), protein (30.6), lactose (48.4), determined using Foss MilkoScan (FT2 Foss, San Angel México) that came from cows treated with drug Ubricina (per 1 ml contains: 100,000 U.I. penicillin G procaine, 100,000 U.I. penicillin G benzathine, 120 mg dihidroestreptomycin sulfate, and 0.2 mg dexamethasone sodium phosphate), in addition to the accumulated non-saleable, transition milk and colostrum. The

raw waste milk was slowly pasteurized in a 1,500 L tank. The milk was heated to 70 °C for 15 sec and then allowed to cool down to 32–38 °C for 30 min before feeding. The waste milk was then stored in a cooling tank for the maximum storage time of 24 h.

The NMR contained (g/kg DM) whey (471.0), concentrated whey protein (52.8), pork fat (266.3), lactose (100.0), soy protein isolate (100.0), lecithin SOLEC 3 F – UB (3.0), vitamins and minerals (0.4), Allura red AC dye (0.5) and sodium butyrate (6.0) plus a probiotic (1.0) of active *B. amyloliquefaciens* at  $1 \times 10^6$  CFU/g DM probiotic. At each feeding, diluted NMR was prepared in steel buckets by mixing 120 g of NMR powder with 1 L of warm water (45 °C). The diluted NMR was then cooled at ambient temperature to 38 °C for 5 min before feeding. The calves were fed individually using buckets, twice daily at 0900 and 1600 h throughout the experimental period according to the following protocol: days 4–20, 2 L liquid; days 21–60, 3 L liquid per feeding per calf. From day 4, calves in both experimental groups were offered pelleted starter *ad libitum* (FOGASA, Aguascalientes, Mexico) once a day in the morning at 0900 h. Freshwater was also offered *ad libitum* to all calves throughout the experiment. Starter feed and water intakes, weight gain and health conditions of individual calves were monitored as described below.

### Measurements and observations

Body weight and height (hoof to the back) were measured respectively using a digital plate scale (Torrey<sup>®</sup> EQM, México), and a wooden ruler (Garrott-Metre<sup>®</sup>, Pro-Vac Inc., Canada) on days 1, 15, 30, 45 and 60 of the experiment. The average daily gain (ADG) was calculated by dividing the BW change by the length of the interval between two consecutive BW measurements (1–15 d, 15–30 d, 30–45 d, and 45–60 d).

Calf health was monitored daily. Feces from individual calf were scored as 1 (normal), 2 (pasty), 3 (loose) and 4 (watery). Rectal temperature was monitored daily at 0900 h and evaluated using a scale: 1 (37.7–38.2 °C), 2 (38.3–38.7 °C), 3 (38.8–39.3 °C) and 4 ( $\geq 39.4$  °C). The appearance of the ears was measured daily during the 60-d period by using a scale: 1 (normal), 2 (non-uniform), 3 (unilateral fall) and 4 (bilateral fall). The eye condition was scored as 0 (normal), 1 (small amount of eye secretion), 2 (moderate amount of secretion in both eyes) and 3 (strong eye secretion). Nasal secretion was also assessed using a scale: 1 (normal), 2 (crystalline mucus), 3 (bilateral secretion) and 4 (purulent mucus secretion). The

presence of a cough was assessed as 1 (not present), 2 (cough reflex), 3 (cough occasional spontaneous) and 4 (repeated coughing). Finally, general health condition was scored as (1) unilateral or bilateral nasal discharge, (2) arthritis or polyarthritis, (3) ear falls (one or two), (4) hirsute or discolored hair, (5) ocular discharge with runoff, (6) abdominal distention, (7) tucked hocks, (8) mutton necked profile, (9) did not meet twice its weight at weaning, (10) reflection of spontaneous or induced cough.

Calves with diarrhea were treated with electrolytes (6 mL/head/d) (Electroidi<sup>®</sup> Investigación Aplicada, S.A. de C.V., México), antibiotics (4 mL/head/d) (3Sulfas<sup>®</sup>, Bio Zoo, S.A de C.V., México) and dewormer (1 g/head/d) (Gisistin<sup>®</sup>, Laboratorios Best, S.A. de C.V., México) according to manufacturer recommendations. The number of days of each calf having diarrhea was also recorded.

### Blood analysis

Venepuncture technique by jugular vein was used to collect 5 mL of blood sample per calf into a vacutainer tube (Vacutainer<sup>®</sup> Becton, Dickinson and Co. Franklin Lakes, NJ, USA), at 0, 24, 48 and 72 h from birth. The blood samples were allowed to stand for 5 min and then centrifuged for 10 min at 4000×g in a clinical centrifuge (Solbat J12<sup>®</sup>, Solbat S.A. de C.V., México). Serum samples were then taken and placed on a refractometer (ATC 311<sup>®</sup>, WorldCom Trade Co., Ltd, China) to measure total serum protein.

### Feed and milk analyses

Starter feed samples were collected weekly and pooled for the analyses of DM, ash, nitrogen (N), crude fat, neutral detergent fibre (NDF) and acid detergent fibre (ADF) in accordance with the procedures of AOAC.<sup>12</sup> Weekly milk samples were collected inside plastic vials of 60 mL (Operadora ETESA S.A. de C.V., Mexico) containing a tablet of conservative (Bronopol, [2-bromo-2-nitro-1, 3 propanediol], Operadora ETESA S.A. de C.V., Mexico). The samples were heated to 40 °C in a water bath and gently mixed before being analyzed in a Milk-o-Scan FT 120 (Foss Analytical Foss Electric, Denmark, Fourier transform infrared) for the contents of fat, crude protein, lactose and total solids. Crude protein in milk was calculated as nitrogen  $\times 6.38$ . The metabolisable energy (ME) contents of PWM and NMR were calculated based on the equations of NRC:<sup>13</sup> Gross energy (GE; Mcal/kg DM) =  $0.057 \times \text{CP}\% + 0.092 \times \text{fat}\% + 0.0395 \times \text{lactose}\%$ ; then ME was calculated as: ME (Mcal/kg DM) =

$GE \times 0.97 \times 0.96$ . To express ME on an MJ per kg basis,  $ME \text{ (MJ/kg DM)} = ME \text{ (Mcal/kg DM)} \times 4.184$ . Further, the ME content of the starter was calculated based on the equations of NRC (2001):  $ME \text{ (Mcal/kg DM)} = 1.01 \times \text{digestible energy (DE; Mcal/kg DM)} - 0.45$ , where  $DE \text{ (Mcal/kg DM)} = 0.04409 \times \text{total digestible nutrient (TDN; \%)} \times 100$ .  $TDN \text{ (\%)} = CP\% + (\text{fat}\% - 1) \times 2.25 + 0.98 (100 - CP\% - \text{Ash}\% - \text{fat}\%) - 7$ . To express ME on an MJ per kg basis,  $ME \text{ (MJ/kg DM)} = ME \text{ (Mcal/kg DM)} \times 4.184$ .

### Economic analysis

Economic analysis was performed using the average market price per liter of waste milk in the region, which was Mexican Peso (MXN\$) 6.5/L. The milk replacer and the starter pellets were bought at MXN\$45.0/kg and MXN\$7.23/kg DM, respectively. The total cost of the feed was calculated as the cost of feeding waste milk or milk replacer, plus the cost of a starter for 60 d. Feed conversion efficiency (kg BW gain/kg feed intake) and economic efficiency (MXN\$/kg BW gain) were also calculated.

### Statistical analysis

Data were analyzed using the PROC MIXED procedure of SAS<sup>14</sup> (version 9.0; SAS Inst. Inc. Cary, NC). Data on BW, intake, ADG, flank height, hip width and serum total protein were analyzed with day as repeated measures. The model included effect of treatment, day, and the treatment  $\times$  day interaction. All the interactions were not significant so only the main effects was reported. Initial BW and flank height were used as covariates to analyze both body weight and flank height, respectively. Two covariance structures were considered in the REPEATED statement in PROC MIXED: compound symmetry (cs) and autoregressive (AR(1)). The error structure with the lowest Akaike information criteria fit statistic was selected for the model. To separate the least square means, the PDIF SAS option was used,  $p < 0.05$  was declared as statistically significant, and trend for  $p \leq 0.10$ .

## Results

### Feed intake and calf performance

Nutrient composition of feeds and feed intake are given in Tables 1 and 2, respectively. The PWM had more CP and crude fat, similar ME content and less lactose content compared with NMR (Table 1). As a result of employing a restrictive liquid feeding

**Table 1.** Chemical composition of experimental diets.

| Composition                                     | Treatments       |                  |                      |
|-------------------------------------------------|------------------|------------------|----------------------|
|                                                 | PWM <sup>1</sup> | NMR <sup>2</sup> | Starter <sup>3</sup> |
| Dry matter (g/kg of fresh weight)               | 118              | 114              | 894                  |
| Components of DM (g/kg of DM)                   |                  |                  |                      |
| Crude protein                                   | 262              | 200              | 189                  |
| Crude fat                                       | 271              | 200              | 78                   |
| Ash                                             | 85               | 42               | 78.2                 |
| Lactose                                         | 356              | 64               | –                    |
| Nitrogen free extract                           | –                | –                | 597                  |
| Neutral detergent fiber                         | –                | –                | 338                  |
| Acid detergent fiber                            | –                | –                | 128                  |
| Metabolizable energy (MJ/kg of DM) <sup>†</sup> | 21.0             | 20.9             | 15.1                 |

<sup>1</sup>PWM: pasteurized waste milk; <sup>2</sup>NMR: non-medicated calf milk replacer containing (g/kg of DM) whey (471), concentrated whey protein (52.8), pork fat (266), lactose (100), soy protein isolate (100), lecithin SOLEC 3F – UB (3.0), vitamins and minerals (0.4), Allura red AC dye (0.5) and sodium butyrate (6.0) plus a probiotic (1.0) of active *Bacillus amyloliquefaciens* at  $1 \times 10^6$  CFU/g DM probiotic; <sup>3</sup>Pelleted starter; <sup>†</sup>Metabolizable energy calculated using NRC (2001) equations.

protocol rather than an *ad libitum* feeding protocol,<sup>3</sup> total liquid intake ( $p = 0.119$ ) was similar between PWM or NMR-fed calves during first 60 days of age (Table 2). Although *ad libitum* amounts of starter pellet were offered to all calves, the total solid feed intake ( $p = 0.547$ ) was also similar between PWM and NMR groups. In addition, there was no difference ( $p = 0.685$ ) between the treatments in metabolizable energy intake. Growth performance and outcomes of the economic evaluation are given in Table 3. Overall, ADG and feed conversion efficiency (kg BW gain/kg feed intake) were similar ( $p > 0.05$ ) between calves receiving PWM and NMR during first 60 days of age (Table 3). Birthweights were similar between PWM and NMR at about 41 kg ( $p = 0.419$ ). Calves receiving PWM tended to have greater BW on day 60 ( $p = 0.056$ ). Average daily gain, flank height, hip width and FCE were not significantly different ( $p > 0.05$ ) between the two calf groups. The economic evaluation showed that the costs of liquid feed intake and both liquid and starter feed intake during the first 60 days of life were 8–9% higher ( $p < 0.001$ ) for the PWM calves than the NMR calves (Table 3). No difference was observed for economic efficiency, expressed as cost per kg of BW gain, between the two calf groups.

### Health condition

Colostrum IgG, serum total protein, and the health scores are given in Table 4. Both PWM and NMR calves received colostrum with similar IgG concentrations ( $p = 0.890$ ). Serum total protein concentrations were also similar between the two calf groups ( $p > 0.459$ ). Scores for nose secretion, eye appearance, ear appearance, and rectal temperature, and general health condition were similar for the two calf groups

**Table 2.** Dry matter and metabolizable energy (ME) intake of Holstein calves fed pasteurized waste milk (PWM) or a non-medicated milk replacer (NMR) for 60 d ( $n = 9$ ).

|                                                     | Least square means |                  |      | <i>p</i> -Value |
|-----------------------------------------------------|--------------------|------------------|------|-----------------|
|                                                     | PWM <sup>1</sup>   | NMR <sup>2</sup> | SEM  |                 |
| <b>Starter intake</b>                               |                    |                  |      |                 |
| DMI from days 4–60 (g of DM/calf/d)                 | 196                | 163              | 38.6 | 0.548           |
| Total intake during 60 d (kg of DM/calf)            | 11.7               | 9.7              | 2.30 | 0.547           |
| Total liquid intake during 60 d (kg of DM/calf)     | 39.4               | 40.0             | 0.26 | 0.119           |
| Starter + liquid intake during 60 d (kg of DM/calf) | 51.1               | 49.7             | 2.39 | 0.686           |
| Total ME intake (MJ/calf/d)                         | 16.8               | 16.4             | 0.61 | 0.685           |

<sup>1</sup>PWM: pasteurized waste milk; <sup>2</sup>NMR: Non-medicated calf milk replacer containing (g/kg of DM) whey (471), concentrated whey protein (52.8), pork fat (266), lactose (100), soy protein isolate (100), lecithin SOLEC 3F – UB (3.0), vitamins and minerals (0.4), Allura red AC dye (0.5) and sodium butyrate (6.0) plus a probiotic (1.0) of active *Bacillus amyloliquefaciens* at  $1 \times 10^6$  CFU/g DM probiotic.

**Table 3.** Body measurements, feed efficiency and economical evaluation of Holstein calves fed pasteurized waste milk (PWM) or a formulated *non-medicated* milk replacer (NMR) for 60 d (means,  $n = 9$ ).

|                                                                 | Least square means |                  |        | <i>p</i> -Value |
|-----------------------------------------------------------------|--------------------|------------------|--------|-----------------|
|                                                                 | PWM <sup>1</sup>   | NMR <sup>2</sup> | SEM    |                 |
| Body weight (kg/calf)                                           | 52.1               | 47.2             | 1.61   | 0.056           |
| Average daily gain (g/calf/d)                                   | 382                | 289              | 56.9   | 0.266           |
| Flank height (cm/calf)                                          | 87.6               | 86.6             | 0.56   | 0.255           |
| Hip width (cm/calf)                                             | 87.1               | 87.4             | 1.34   | 0.897           |
| FCE3 (kg BW gain/kg of DM feed intake)                          | 0.425              | 0.350            | 0.0570 | 0.367           |
| <b>Economic evaluation during 60 d in Mexican Peso (MXN \$)</b> |                    |                  |        |                 |
| Cost of liquid intake                                           | 2013               | 1840             | 12.7   | <0.001          |
| Cost of liquid and starter intake                               | 2298               | 2129             | 14.6   | <0.001          |
| Economic efficiency (/kg of BW gain)                            | 158                | 141              | 39.1   | 0.760           |

<sup>1</sup>PWM: pasteurized waste milk; <sup>2</sup>NMR: non-medicated calf milk replacer containing (g/kg of DM) whey (471), concentrated whey protein (52.8), pork fat (266), lactose (100), soy protein isolate (100), lecithin SOLEC 3F – UB (3.0), vitamins and minerals (0.4), Allura red AC dye (0.5) and sodium butyrate (6.0) plus a probiotic (1.0) of active *Bacillus amyloliquefaciens* at  $1 \times 10^6$  CFU/g DM probiotic; <sup>3</sup>FCE: feed conversion efficiency.

( $p > 0.05$ ). However, the NMR calves had a higher fecal score ( $p < 0.001$ ) and a lower cough score ( $p < 0.012$ ) than the PWM calves (Table 4).

## Discussion

### Feed intake and calf performance

While this study showed no difference in ADG, it is interesting to note that previous research comparing feeding NMR with liquid milk showed inconsistent results pertaining to calf growth rates. For instance, Brown et al.<sup>15</sup> and Kmicikewycz et al.<sup>16</sup> reported higher growth rates for feeding NMR than feeding waste milk, whereas Godden et al.<sup>2</sup> and Lee et al.<sup>3</sup> found that calves fed pasteurized waste milk or whole milk grew faster and were heavier at weaning compared to calves fed NMR. In the current study, both liquid and starter feed had adequate ME and protein contents to support calf growth according to NRC<sup>13</sup> recommendations. Owing to similar liquid and solid feed intake, PWM- and NMR-fed calves achieved

similar ME intake, which is consistent with the previous observations by Kazemi-Bonchenari et al.<sup>17</sup> Moreover, the overall liquid and starter feed intake were similar during the 60-day period. Therefore, both PWM and NMR calves achieving similar ADG could be primarily due to a similar amount of nutrient supply for tissue growth. Furthermore, the lack of difference in feed conversion efficiency might also have contributed to the lack of difference in ADG, as both groups were equally efficient in turning feed into tissue gain. Nonetheless, calves receiving PWM consumed a significantly greater amount of starter feed during days 16 and 45 but it was not translated into ADG, suggesting that a considerable amount of nutrients could be partitioned away from growth to other functions in PWM-fed calves. Given the fact that neonate calves are under considerable risk of immune challenges such as diarrhea and respiratory diseases, more nutrients could be partitioned for use by the immune system. In the present study, feeding PWM or NMR appeared to differentially affect diarrhea and respiratory disorders as PWM-fed calves had

**Table 4.** Clinical and health conditions evaluation of holstein calves fed pasteurized waste milk (PWM) or a non-medicated milk replacer (NMR) for 60 d ( $n = 9$ ).

|                                                     | Treatments       |                  | SEM   | p-Value |
|-----------------------------------------------------|------------------|------------------|-------|---------|
|                                                     | PWM <sup>1</sup> | NMR <sup>2</sup> |       |         |
| Colostrum IgG <sup>3</sup> (mg/mL)                  | 77.5             | 78.8             | 6.29  | 0.890   |
| Serum total protein (mg/dL)                         | 5.48             | 5.31             | 0.166 | 0.459   |
| Days of diarrhea treatment                          | 2.88             | 3.00             | 0.160 | 0.590   |
| Faeces score <sup>4</sup> (1 to 4)                  | 1.44             | 2.55             | 0.126 | <0.001  |
| Rectal temperature score <sup>5</sup> (from 1 to 4) | 3.09             | 2.92             | 0.158 | 0.476   |
| Cough score <sup>6</sup> (from 1 to 4)              | 1.61             | 1.09             | 0.128 | 0.012   |
| Nose secretion score <sup>7</sup> (from 1 to 4)     | 1.11             | 1.05             | 0.034 | 0.191   |
| Eye score <sup>8</sup> (from 0 to 3)                | 1.00             | 1.00             | 0.003 | 1.000   |
| Ear score <sup>9</sup> (from 1 to 4)                | 1.00             | 1.00             | 0.003 | 1.000   |
| General health condition score <sup>10</sup>        | 9.55             | 9.77             | 0.108 | 0.177   |

<sup>1</sup>PWM: pasteurized waste milk; <sup>2</sup>NMR: non-medicated calf milk replacer containing (g/kg of DM) whey (471), concentrated whey protein (52.8), pork fat (266), lactose (100), soy protein isolate (100), lecithin SOLEC 3F – UB (3.0), vitamins and minerals (0.4), Allura red AC dye (0.5) and sodium butyrate (6.0) plus a probiotic (1.0) of active *Bacillus amyloliquefaciens* at  $1 \times 10^6$  CFU/g DM probiotic; <sup>3</sup>IgG: immunoglobins G; <sup>4</sup>Faeces score (1 = normal, 2 = pasty, 3 = semi, 4 = liquids); <sup>5</sup>Rectal temperature score (1 = 37.7–38.2 °C, 2 = 38.3–38.7 °C, 3 = 38.8–39.3 °C, 4 > 39.4 °C); <sup>6</sup>Cough score (1 = not present, 2 = cough reflex, 3 = cough occasional spontaneous, 4 = repeated coughing); <sup>7</sup>Nose secretion score (1 = normal, 2 = crystalline mucus, 3 = bilateral secretion, 4 = purulent mucus secretion); <sup>8</sup>Eye score (0 = normal, 1 = small amount of eye secretion, 2 = moderate amount of secretion in both eyes, 3 = strong eye secretion); <sup>9</sup>Ear score (1 = normal, 2 = non-uniform, 3 = unilateral fall, 4 = bilateral fall); <sup>10</sup>General condition and health score (from 1 to 10).

more solid feces, whereas NMR-fed calves had less coughing. Wanat et al.<sup>18</sup> observed that supplementation of sodium butyrate in starter feed linearly increased fecal fluidity as the dose increased. However, the number of days with diarrhea in that study did not increase linearly with the dose, indicating that feeding sodium butyrate could be related to watery feces independent of diarrhea.

It is important to point out that the ADG was 32% higher for the PWM calves than the NMR calves particularly during days 16 and 30. We speculate that the large sample variation within each treatment group may have reduced the chance that the difference being statistically significant. Therefore, a greater number of animals per treatment group (e.g.,  $n > 9$ ) would have demonstrated a statistically significant impact of PWM on ADG compared to NMR. The PWM was a combination of non-saleable milk, milk from transition cows, and colostrum, likely having high concentrations of IgG, immunoglobins M (IgM) and immunoglobins A (IgA) providing additional immune support and thus promoting growth prior to weaning.<sup>19</sup> Consistent with the observation by Lee et al.,<sup>3</sup> we did not observe differences in rectal temperature and respiratory (e.g., nasal and ear) scores. Overall, the general health condition of calves in the present study was satisfactory and there was no difference

between PWM and NMR groups. This could be partly a result of adequate nutrient supply to the calves.<sup>2</sup> The NMR would result in notable health benefits if calves were under stresses such as negative energy balance.<sup>20</sup>

In line with feed intake and body weight, the flank height of calves fed PWM were numerically greater than that of NMR fed calves at day 45 but became similar between the groups by day 60. So, another important observation we made in this study was that the numerical differences in feed intake and body weight between PWM and NMR became smaller as calves got older (>45 d). This suggests that the anticipated benefits of sodium butyrate and probiotic supplementation on rumen development and health might require time to be reflected in the calf performance. Therefore, examination of calves for a longer period than the current experimental period (60 days of age) could reveal improved performance for supplementation of probiotics and sodium butyrate in milk replacer compared to feeding fluid milk.

### Economic evaluation

Reduction of feeding cost and thus increasing economic gain are the main reasons for feeding milk replacers because they are composed primarily of less costly ingredients than fluid milk. The present study results show that rearing calves on NMR was more cost-effective than rearing them on PWM in terms of total feed cost, the major part of the total cost. This finding is in contrast with the results from previous work. Godden et al.<sup>2</sup> found that the estimated savings on feeding pasteurized waste milk, compared with commercial milk replacer, was US\$0.69/calf per day. El-Jack and Ahmed<sup>21</sup> reported that the cost of rearing dairy calves on milk replacer was significantly less than rearing them on raw milk. However, the economic efficiency was similar between the two groups in the current study. There are two potential reasons which may help explain the lack of difference in the economic efficiency. Unlike Brown et al.<sup>15</sup> and Kmicikewycz et al.<sup>16</sup> which reported improved growth rate with conventional milk replacer than with waste milk but similar ADG, greater growth rate and ADG were noted with PWM in the present study. In addition, the increased cost of feeding PWM may have been offset by the numerically higher ADG from PWM, resulting in no net gain in economic efficiency.

## Conclusions

Calves receiving PWM ate more and had greater body weights than calves receiving NMR when they were 2–6 weeks of age. However, the differences disappeared as the calves approached 9 weeks of age. Overall, the ADG and general health scores were similar between PWM and NMR-fed calves during the first 60 days of their lives. Therefore, non-medicated milk replacers fortified with sodium butyrate and *Bacillus* probiotics may be used as a replacement for PWM without having a negative impact on calf growth rate and health status.

## Availability of data and materials

The datasets generated or analyzed during the current study are available from the first author (H.E.M. Kamel) on reasonable request. The datasets supporting the conclusions of this article are included within the article.

## Ethical approval

All animal experiments were reviewed and approved by the Institutional Animal Care and performed of the Autonomous University of Aguascalientes in accordance with the ethical standard laid down in the 1996 declaration of Helsinki and its later amendments and with the Guidelines for Experimental Animals.

## Disclosure statement

The authors declare that they have no competing interests.

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