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Short communication: Effects of porcine plasma or combined sodium butyrate and *Bacillus subtilis* on growth and health of grain-fed veal calves

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ABSTRACT

The objective of this study was to evaluate the effects of incorporating 2 commonly used additives or spray-dried porcine plasma in calf milk replacer (CMR) on calf performance and health. Male Holstein calves (n = 158) transported from auction barns and local dairy farms were randomly assigned to receive 1 of 3 decoquinate-containing CMR for the first 49 d of the experiment: all milk protein and no additives (CONT); 15% of crude protein (CP) replaced with spray-dried porcine plasma, no additives (PLM); or all milk protein and an added combination of sodium butyrate (rate 1.4 kg of butyric acid/Mt) and *Bacillus subtilis* (1.28 million cfu/g of feed; BB). All milk replacers were formulated to contain 26% CP and 17% fat, 2.4% Lys, and 0.8% Met, and were bucket-fed at daily feeding rates of 520 g during wk 1 and 2, 650 g during wk 3, and 900 g during wk 4 and 5, in a total of 4, 5, and 6 L of solution, respectively. Calves were offered texturized calf starter (18% CP) upon arrival until wk 3 and transitioned to a corn and pellet ration with 2% straw (18.1% CP). No prophylactic administration of antibiotics occurred. All calves were gradually weaned over a 2-wk period. Calves were individually housed until weaned and then housed in groups of 5 in a mechanically ventilated facility in southwestern Ontario, Canada. Fecal scores, treatments administered (antibiotic or supportive therapy), and mortalities were recorded daily. Body weight was measured using a digital scale at arrival and at 14, 49, 56, and 78 d after arrival. No differences were found among the groups with respect to growth, feed efficiency, or incidence of diarrhea or respiratory infection treatment. Calves supplemented with BB had a greater hazard of mortality over the growing period compared with CONT. An interaction was found between the BB group and the level of total serum protein, with the BB group having a lower proportion of days with a fecal score of 3 when the calves had a higher total serum protein level. Calves fed PLM had a lower proportion of d with a fecal score of 3 relative to CONT but no difference in the proportion of d with a fecal score of 2 or higher. This study found that the addition of spray-dried plasma in CMR reduced diarrhea severity; however, supplementing BB was associated with a higher hazard of calf mortality and had a varying response on fecal score.
Key words: dairy calf, calf milk replacer, spray-dried plasma, direct-fed microbial

Short Communication

Antimicrobials have traditionally been provided to young dairy calves to improve their health and growth. This is particularly true in the veal sector, where group oral antimicrobial therapy at arrival remains a standard practice (Pardon et al., 2012; Jarrige et al., 2017). This high level of antimicrobial use in the veal calf sector (Bos et al., 2013) has been associated with the development of antimicrobial resistance in commensal bacteria and animal pathogens (Catry et al., 2016). With little evidence available to support the use of oral group antimicrobial therapy, it would be judicious to explore alternatives to this strategy, as it represents the majority of antimicrobial consumption (Pardon et al., 2012; Jarrige et al., 2017).

Spray-dried plasma added to calf milk replacer (**CMR**) could be one such alternative. It has been demonstrated to improve weight gain, hydration, and attitude in calves challenged with enterotoxigenic *Escherichia coli* (Quigley and Drew, 2000) and to reduce calf mortality, improve DMI, and reduce diarrhea (Quigley and Wolfe, 2003). Porcine plasma is derived from whole blood collected in USDA-APHIS–inspected abattoirs and contains significant concentration of immunoglobulins (16 to 22.5% on spray-dried air-dry

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basis; Pierce et al., 2005), growth factors (Louveau et al., 1991), and various functional proteins, which could improve intestinal health. Spray-dried plasma contains 78% CP (Nutrapro, APC Inc., Ankeny, Iowa) and has been evaluated for use as an alternative to antimicrobials in diets for weanling pigs (Torrallardona, 2010) and to reduce mortality and morbidity in dairy calves (Thornsberry et al., 2016).

Supplementation with *Bacillus*-based products has also been shown to affect calves. The addition of a *Bacillus*-based direct-fed microbial to an electrolyte administered to scouring calves was associated with a reduced fecal shedding of *Clostridium perfringens* and reduced severity of the diarrhea event, compared with administration of electrolyte alone (Wehnes et al., 2009). Jenny et al. (1991) also found that continuously fed *Bacillus subtilis* via CMR powder showed a trend toward improved preweaning feed conversion and a trend toward increased ADG postweaning.

Butyric acid (C4:0) is reported to contain between 3.26 and 4.73 g/100 g fatty acids in whole milk, depending on the feedstuffs fed (Schroeder et al., 2003). However, in commercial milk replacers, many of the ingredients, such as lard, tallow, palm and coconut oil, and other fats and oils, are void of butyric acid. Research has demonstrated that supplementing butyric acid in commercial milk replacers has led to improved ADG and feed conversion in veal calves (Guilloteau et al., 2009), increased digestibility and higher pancreatic secretions (Guilloteau et al., 2010), and improved rumen development (Gorka et al., 2009) in calves. Thus, the addition of butyric acid could lead to an economic advantage for producers rearing calves.

The objective of this study was to compare the effects of the addition of several commercial additives commonly used in Ontario with the use of spray-dried porcine plasma in CMR on calf health and growth. We hypothesized that inclusion of the additives or spray-dried plasma would lead to improvements in growth and reductions in levels of diarrhea.

The randomized clinical trial was conducted between April 30 and August 7, 2018, at Mapleview Agri Ltd., a grain-fed veal facility used for commercial milk replacer and additive research, located in southwestern Ontario, Canada. This facility was selected due to availability of research technicians and ability to monitor the outcomes of the trial. Calves were sourced from local dairy farms in Ontario or auction facilities in Ontario and Quebec. Calves arrived at the facility in 2 batches of approximately 80 each, delivered 3 wk apart.

Calves were randomized into blocks of 10 according to a randomization command in Microsoft Excel (Microsoft Corp., Redmond, WA) and allotted to 1 of 3 CMR treatments: all milk protein and no additives (**CONT**), 15% of crude protein replaced with spray-dried porcine plasma and no additives (**PLM**), or all milk protein and an added combination of sodium butyrate (70% butyric acid, Proformix, Probiotech, Saint Hyacinthe, Québec, Canada; rate 2 kg/Mt) and *Bacillus subtilis* (1.28 million cfu/g of feed, BioPlus 2B, Chr. Hansen Inc., Milwaukee, WI; 400 g/Mt; **BB**). The researchers were responsible for allocation to the treatment groups. Milk replacers (Mapleview Milk Replacer, Mapleview Agri Ltd., Palmerston, Ontario, Canada) were blended to include the ingredients for each treatment group before the start of the study. The observers, producer, and person responsible for data analysis were blinded to the treatment groups.

Blood samples were taken upon enrollment in the study, and a digital refractometer was used to determine total serum protein. Total serum protein of <5.1 g/dL was used as the threshold for determination of failure of passive transfer of immunity (Renaud et al., 2018).

Calves were individually housed in 1-m^2 individual stalls for the milk feeding period and were put into groups of 5 calves at weaning. All CMR contained decoquinate (30 mg/kg) and were formulated to contain 26% CP, 17% fat, 2.4% Lys, 0.8% Met, and 1.6% Thr. All were bucket-fed at daily feeding rates of 520 g for wk 1 and 2, 650 g for wk 3, and 900 g for wk 4 and 5, in a total of 4, 5, and 6 L of solution, respectively, with feedings split evenly between a.m. and p.m. No group medications, including antimicrobials, were administered. All calves were weaned over a 2-wk period by feeding 450 g of CMR once daily during wk 6 and 450 g of CMR every third feeding during wk 7. Calves were provided with free-choice water for the entire experiment and were offered texturized calf starter (18% CP) upon arrival until wk 3, before transitioning to corn and pellet ration with 2% straw (18.1% CP) for the remainder of the trial (both grain mixes manufactured by Wallenstein Feed and Supply Ltd., Wallenstein, Ontario, Canada).

Body weight was recorded at arrival and at 14, 49, 56, 63, and 78 d after arrival, using a digital scale. Grain was weighed back on d 7, 14, 21, 28, 49, and 78 after arrival, with all new grain added being weighed. Milk refusals were recorded twice daily, after milk feeding. Feed efficiency was calculated for the entire experimental period (0 to 78 d after arrival), preweaning period (0 to 49 d after arrival), and postweaning period (49 to 78 d after arrival), using the total amount of feed consumed (milk replacer and concentrate) on an as-fed basis divided by the number of kilograms gained. We calculated feed consumption on an as-fed basis because specific dry matter values were not available for all feeds consumed in the experiment.

Calves were scored daily for fecal consistency for the first 28 d of the experimental period. Fecal score 0 = normal consistency; 1 = semi-formed or pasty; 2 = loose feces; 3 = watery feces (McGuirk, 2008). The proportion of days with an abnormal fecal score of 2 or 3 was calculated by dividing the number of days with an abnormal fecal score by the number of days under observation. Antibiotic and supportive treatments were recorded when administered to each calf.

The number of calves enrolled was based on the availability of calves and resources required to complete this study. Hence, no formal sample size calculation was made to establish the number of calves enrolled in this study.

All statistical analyses were conducted in Stata 14 (StataCorp, College Station, TX). Descriptive statistics were generated on all explanatory variables in the data set. Differences in the means of continuous and normally distributed explanatory variables among treatment groups were evaluated using *t*-tests, and the means of non-normally distributed continuous variables were evaluated using Wilcoxon rank-sum tests. Differences between frequency counts in categories of categorical variables were evaluated using a chi-squared test, with a P-value < 0.05 indicating significant difference.

Linear regression models were created to evaluate the effect of treatment group on ADG and feed efficiency at 14, 49, and 78 d after enrollment. To evaluate the proportion of d at risk, with a fecal score of 2 or 3, a generalized linear model with a logit link and binomial family was used. Last, a Cox proportional hazards model was created to evaluate the effect of treatment group on mortality and on treatment for diarrhea and pneumonia during the experimental period. Univariable regression models were constructed to screen for variables that were unconditionally associated with outcomes, using a liberal P-value of 0.2. Risk factors with univariate associations ($P < 0.2$) were subsequently offered to a multivariable model, through a manual backward stepwise process (Dohoo et al., 2010). Confounding factors were assessed through evaluation of the effects of the removed variables on the coefficients of the remaining variables. A variable was deemed a confounder if it was not an intervening variable, based on the causal diagram, and if the coefficient of a significant variable in the model changed by at least 20% with its removal. Two-way interactions were evaluated between biologically important variables and remained in the final models if significant ($P < 0.05$). If variables did not meet the assumption of linearity, they were categorized into quartiles. For each model, model assumptions were evaluated for model fit, and outliers were identified and explored.

A total of 158 calves were enrolled in the trial, with 53 calves each randomly assigned to the control or the BB groups and 52 calves randomly assigned to the PLM group. The mean weight of the calves at arrival was 47.6 kg; no statistical differences were found among the treatment groups using a *t*-test ($P = 0.56$). The average total serum protein level was 5.69 g/dL, with 18% of calves having failure of passive transfer of immunity. The level of total serum protein ($P = 0.48$) was not statistically different among the groups using a *t*-test; however, the incidence of failure of passive transfer of immunity tended to be higher in the control group ($P = 0.07$) using a chi-squared statistic. A total of 61 calves were sourced from an auction facility, with 18, 23, and 20 of these calves sorted into the CONT, PLM, and BB groups, respectively. This was not statistically different among groups ($P = 0.61$).

A total of 23 calves (14.5%) died during the trial, with 7.5, 11.5, and 24.5% dying in the CONT, PLM, and BB groups, respectively. The Cox proportional hazards model found that a calf arriving with a higher total serum protein tended to have a reduced hazard of dying in the growing period [hazard ratio (**HR**): 0.47; 95% CI: 0.21 to 1.03; $P = 0.06$], and calves arriving from Quebec auction facilities had a lower hazard of dying (HR: 0.21; 95% CI: 0.05 to 0.96; $P = 0.04$). Calves in the BB group had a greater hazard of mortality over the growing period compared with the CONT group (HR: 3.78; 95% CI: 1.19 to 11.91; $P = 0.02$). Of the calves that died, 4 died from diarrhea, 17 from pneumonia, and 2 from dehydration, as determined by facility staff.

Calves spent 29.3, 28.6, and 28.0% of the first 28 d with a fecal score of 2 or higher in the CONT, PLM, and BB groups, respectively. Neither the BB group nor the PLM group was associated with the proportion of days with a fecal score of 2 or higher. However, Ontario auction calves (relative proportion ratio: 1.56; 95% CI: 1.24 to 1.95; $P < 0.001$) and the second group of approximately 80 calves delivered 3 wk later (relative proportion ratio: 1.25; 95% CI: 1.02 to 1.53; $P = 0.01$) had a greater proportion of days with a fecal score of 2 or higher. Calves with total serum protein of 5.65 to 6.10 mg/dL (relative proportion ratio: 0.75; 95% CI: 0.59 to 0.97; $P = 0.03$) had lower proportions of days with a fecal score of 2 or higher compared with calves with total serum protein <5.2 mg/dL.

Calves had a fecal score of 3 for 13.1, 10.7, and 14.9% of the first 28 d in the CONT, PLM, and BB groups, respectively. The PLM group had a lower proportion of time with a fecal score of 3 compared with the control group, whereas the BB group had a greater proportion of time with a fecal score of 3 compared with the con-

Table 1. Generalized linear regression model evaluating proportion of scoring periods with a fecal score of 3 in the first 28 d following arrival

Variable	Category	Relative proportion ratio	Coefficient	SE (coefficient)	*P*-value	95% CI (coefficient)
Treatment group[1]	CONT	Referent				
	PLM	0.55	−0.59	0.27	0.03	−1.11 to −0.07
	BB	2.10	0.74	0.27	0.006	0.21 to 1.27
Total protein	<5.2 g/dL	Referent				
	5.2 to 5.65 g/dL	0.91	−0.09	0.31	0.78	−0.70 to 0.52
	5.65 to 6.1 g/dL	0.75	−0.29	0.25	0.25	−0.78 to 0.20
	≥6.1 g/dL	0.92	−0.08	0.26	0.75	−0.60 to 0.43
Interaction term[2]	BB × 5.2 to 5.65 g/dL	0.30	−1.19	0.45	0.008	−2.06 to −0.32
	BB × ≥6.1 g/dL	0.26	−1.35	0.42	0.001	−2.17 to −0.52
Source	Ontario	Referent				
	Auction	1.51	0.41	0.16	0.009	0.10 to 0.72
	Quebec drover	0.89	−0.11	0.20	0.56	−0.52 to 0.28
Trial group	1	Referent				
	2	1.39	0.33	0.14	0.01	0.07 to 0.60
Constant		0.12	−2.06	0.22	0.0001	−2.49 to −1.63

[1]CONT = all milk-protein milk replacer, no additives; PLM = 15% of crude protein in milk replacer substituted with spray-dried porcine plasma, no additives; BB = all milk protein with a combination of sodium butyrate (70% butyric acid) and *Bacillus subtilis* (1.28 million cfu/g) added.
[2]Only statistically significant interactions are presented.

trol group (Table 1). We found an interaction between treatment group and level of total serum protein, with the BB group having a lower proportion of days with a fecal score of 3 when calves had a higher total serum protein level. Source and trial group were both associated with the outcome (Table 1).

Overall, 136 calves (86.1%) were treated (meloxicam and trimethoprim sulfa) at least once for diarrhea, with 91% of the CONT group, 88% of the PLM group, and 79% of the BB group being treated. Survival analysis was conducted and found no statistical differences among the treatment groups with respect to diarrhea treatment; source of the calves was the sole variable associated with diarrhea treatment, with Ontario (HR: 1.58; 95% CI: 1:07 to 2.33; $P = 0.02$) and Quebec (HR: 1.71; 95% CI: 1:09 to 2.68; $P = 0.02$) auction-derived calves having greater risk of being treated for diarrhea compared with calves sourced from local Ontario dairy farms.

A total of 152 calves (96.2%) were treated once for respiratory disease during the experimental period. In the CONT group, 96% of the calves were treated; 96% of the calves in the PLM group were treated; and 94% of calves in the BB group were treated. No differences were found with a chi-squared test ($P = 0.61$) or in the Cox proportional hazards model.

Calf body weights, ADG, and feed efficiency measured over the course of the experiment are reported in Table 2. Body weights and ADG were not significantly different by treatment groups at any time point when using a *t*-test. Feed efficiency was not found to be statistically different by treatment groups using a Wilcoxon rank-sum test.

Linear regression models were built for the ADG calculated for the preweaning (0 to 49 d after arrival), postweaning (49 to 78 d after arrival), and entire experimental periods. In the preweaning period, treatment group had no effect on growth; however, we found that calves from an Ontario auction had 0.11 kg/d (95% CI: −0.19 to −0.06 kg/d; $P = 0.01$) reduction in growth compared with calves sourced from local Ontario dairy farms. None of the measured variables had a significant effect on ADG postweaning or over the entire experimental period.

Feed efficiency was also evaluated using linear regression models; however, as none of the initial linear mixed models met the assumption of normality, feed efficiency had to be inversely transformed. No associations were found between the variables of interest and feed efficiency over the preweaning, postweaning, or entire experimental period.

The addition of spray-dried plasma to CMR has been shown to reduce the incidence and severity of diarrhea (Quigley and Drew, 2000; Thornsberry et al., 2016), and the results of our study further augment these findings. The addition of spray-dried plasma to CMR has also been shown to reduce calf mortality (Quigley and Drew, 2000; Quigley and Wolfe, 2003); however, despite experiencing total mortality of 14.5% in the study, indicating significant disease challenge, inclusion of spray-dried porcine plasma in CMR did not reduce calf mortality (11.5%). However, it is important to note that 73.9% of the mortality recorded was due to respiratory disease, which was not reduced by the spray-dried plasma in this study. Further research is needed to examine the effects of feeding spray-dried plasma to

Table 2. Mean (SE) for BW, ADG, and feed efficiency, presented by treatment group

Treatment group[1]	BW [kg (SE)]			ADG [kg/d (SE)]			Feed efficiency [kg of feed per kg of gain (SE)]		
	Day 0	Day 49	Day 77	Day 0 to 49	Day 0 to 77	Day 49 to 77	Day 0 to 49	Day 0 to 77	Day 49 to 77
CONT	48 (0.45)	75 (1.86)	108 (3.85)	0.55 (0.04)	0.76 (0.05)	1.08 (0.09)	2.84 (0.30)	2.93 (0.34)	2.25 (0.33)
PLM	48 (0.48)	75 (1.46)	111 (3.07)	0.55 (0.03)	0.82 (0.04)	1.25 (0.09)	2.64 (0.21)	2.90 (0.66)	1.66 (0.64)
BB	48 (0.49)	77 (1.73)	111 (3.03)	0.61 (0.03)	0.81 (0.04)	1.11 (0.08)	2.34 (0.17)	2.66 (0.30)	2.04 (0.57)

[1]CONT = all milk-protein milk replacer, no additives; PLM = 15% of CP in milk replacer substituted with spray-dried porcine plasma, no additives; BB = all milk protein with a combination of sodium butyrate (70% butyric acid) and *Bacillus subtilis* (1.28 million cfu/g) added.

respiratory disease–challenged calves. Our study also suggests that spray-dried porcine plasma is a suitable replacement for whey-based proteins in CMR. When replacing 15% of the skim milk–based protein with spray-dried plasma, no differences were found with respect to growth over the entire experimental period.

Continuous feeding of *Bacillus subtilis* for 42 d in CMR has been shown to increase the release of acute-phase proteins and activated B cells in dairy calves (Duersteler et al., 2012). However, in our study, this group had little effect on health except for a reduced proportion of days with a fecal score of 3, if the calf had a total serum protein of greater than 6.1 mg/dL. The BB group also had a greater risk of mortality throughout the experimental period. It could be hypothesized that some calves fed *Bacillus subtilis* could have experienced excessive stimulation of immune function when both challenged with severe respiratory disease and continuous exposure to *Bacillus subtilis*, resulting in a significant increase in calf mortality (24.5%), coupled with an increase in days with a fecal score of 3. We hypothesize that severely challenged male dairy calves transported from auctions, comingled and housed in barns, and experiencing poor colostrum status may be harmed by excessive and continuous immune stimulation associated with supplementation of *Bacillus subtilis*.

The addition of butyric acid to calf milk replacer has not been associated with increased calf mortality or increased immune stimulation or immune function; rather, research shows improved ADG and feed conversion in veal calves using a butyric acid supplement (Guilloteau et al., 2009), increased digestibility and pancreatic secretions (Guilloteau et al., 2010), and improved rumen development (Gorka et al., 2009). However, in this study, the addition of this product did not lead to improved ADG or improved feed conversion when combined with *Bacillus subtilis*. This lack of effect could be due to the overwhelming respiratory infection pressure that was present during this experiment or to a possible negative interaction between the addition of butyric acid and *Bacillus subtilis*.

In summary, addition of spray-dried plasma to CMR reduced incidence of severe diarrhea and had no effect on ADG or incidence of medical treatment, whereas supplementing BB increased hazard of calf mortality.

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