Impact of NutraGlo on Growth and Health of Holstein Calves: A Randomized Clinical Trial

Final Report

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EXECUTIVE SUMMARY

- No statistical differences existed between the groups at enrollment, with no statistical differences between weight at arrival and failure of passive transfer (**FPT**) status between the treatment groups.

- The Nutra-Glo (**NG**) group had a 28% reduction in the relative proportion of days with a fecal score of ≥ 2 and a 51% reduction in the relative proportion of days with a fecal score of 3.

- NG group had significantly lower odds of being treated with antibiotics for diarrhea. No differences between the groups was found with respect to treatment for respiratory disease.

- The average daily gain (**ADG**) from 0 to 78 days found that the NG group had a 0.29 lb/d increase. The growth advantage was mostly seen in the post weaning period with a trend for a 0.57 lb/d increase in ADG from 49 to 78 days after arrival.

- The feed efficiency for the entire experimental period was improved in the NG group using a basic statistical test. However, as the data were not normally distributed, a proper statistical model could not be built.

OBJECTIVES

The objective of this project was to assess the efficacy of NutraGlo on reducing the incidence of calf diarrhea when fed at arrival to a veal facility. A secondary objective was to assess the impact that feeding NutraGlo had on growth during the production period.

METHODS

This randomized clinical trial was conducted at a grain-fed veal research facility (Mapleview Agri Ltd.) within the southwestern region of the province of Ontario, Canada. Upon arrival to the facility, calves were administered an intranasal bacterin (Once PMH® IN (for control of *Mannheimia haemolyitca* and *Pasteurella multocida*)) and viral vaccine (Bovilis® IN (for control of enteric disease caused by coronavirus)). The calves were re-vaccinated with a modified-live vaccine (Vista® Once SQ (for control of bovine rhinotracheitis virus, bovine viral diarrhea virus, bovine parainfluenza virus, bovine respiratory syncytial virus, *Mannheimia haemolyitca,* and *Pasteurella multocida*)) at 14- and 28-days following arrival to the facility.

Calves were individually housed for the milk feeding period and subsequently reared in groups of 5 at weaning. They were fed 26% protein and 17% fat milk replacer using the following schedule: 260 g in 2 L twice daily for week 1 and 2; 350 g in 2 L twice daily for week 3; 425 g in 3 L twice daily for week 4 and 5; and 425 g in 3 L once daily for week 6 and 7. The calves were offered texturized calf starter (18% CP) upon arrival until week 3 and transitioned to corn and pellet ration with 2% straw (18.1% CP) for the remainder of the experimental period. NutraGlo (**NG**) was administered in the tank mix of milk at 5 ml / calf for week 1 and 2 and at 2 ml / calf from week 3- 7. In the post-weaning stage weeks 7-11 NG was administered once daily into the grain ration at 2 ml / calf.

Randomization and Blinding

Upon arrival at the veal facility, 80 calves were randomly assigned to 1 of 2 dietary treatments in the room: Control or NG. The dietary treatments were added at each feeding when the milk powder was being mixed. Calves were randomized in blocks of 10 according to a randomization command in Microsoft Excel.

Data Collection

Blood samples were taken once from each enrolled calf at arrival to the veal facility. The blood was spun and a digital refractometer was used to determine serum total protein. A serum total protein of < 5.1 g/dL was used as the threshold for determination of failure of passive transfer of immunity (**FPT**).

Body weight was taken at 14, 49, 56, and 78 days following arrival using a digital scale. Grain was weighed back on days 7, 14, 21, 28, 49, and 78 following arrival with all new grain added being weighed. Milk refusals were recorded twice daily following milk feeding.

Calves were scored twice daily for fecal consistency using the McGuirk (2008) scoring method, where fecal score 0 = normal consistency; 1 = semi-formed or pasty; 2 = loose feces; 3 = watery feces. Calves with a fecal score of 2 or 3 were classified as positive for diarrhea. All antibiotic and supportive treatments were recorded when administered to each calf.

Statistical Analysis

All statistical analyses were conducted in Stata 14 (StataCorp, 2015). Data were imported from Microsoft Excel into Stata 14 and checked for completeness. A causal diagram was created to evaluate the relationships between the potential explanatory variables and the outcomes of interest.

Descriptive statistics were generated on all explanatory variables in the dataset. Differences in the means of continuous and normally distributed explanatory variables between treatment groups were evaluated using t-tests, while 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 (χ^2) with a *P*-value of < 0.05 indicating a significant difference.

Several explanatory multivariable models were created to explore the variables contained within the dataset. Logistic regression models were built to investigate variables associated with antibiotic treatment for diarrhea and respiratory disease. Linear regression models were created to evaluate the impact treatment group had on average daily gain (**ADG**) in the 14, 49, 56, and 78 days following enrollment. To evaluate the proportion of days at risk with a fecal score ≥ 2 or a fecal score of 3, a generalized linear model with a logit link and binomial family was used. Lastly, a Cox proportional hazard model was created to evaluate the impact that treatment group had on mortality in the experimental period and treatment of diarrhea.

The assumption of linearity of continuous variables in the logistic and linear models was assessed by plotting the outcome against the variable of interest. In the Cox proportional hazard model, the assumption of linearity was evaluated by computing the Martingale Residuals and plotting the residuals against the predictor. If a variable failed to meet the linearity assumption, the variable was categorized. In some models, serum total protein and weight at arrival were not linearly associated with the outcome and were categorized into quartiles. Co-linearity among the explanatory variables was tested using Spearman rank coefficients. If the correlation coefficient between 2 variables was ≥ 0.7, only one variable was retained based on fewest missing values, reliability of measurement, and/or biological plausibility.

Univariable regression models were constructed to screen for variables that were unconditionally associated with the outcome using a liberal *P*-value of 0.2. Risk factors that had univariate associations ($P < 0.2$) were subsequently offered to a multivariable model through a manual backward stepwise process. Evaluating the effect of the removed variables on the coefficients of the remaining variables was used to assess confounding. A variable was deemed to be a confounder if it was not an intervening variable, based on the causal diagram, and the coefficient of a significant variable in the model changed by at least a 20%. Two-way interactions were evaluated between biologically important variables and remained in the final models if significant ($P < 0.05$).

For the linear regression models, homoscedasticity and normality were evaluated visually for model fit. Outliers were identified and evaluated using Cook's D, DFITS, and DFBETA. For the logistic models, fit was assessed using Pearson and Deviance goodness of fit tests. Outliers were identified and evaluated using residuals calculated for each model. The assumption of proportionality was assessed for the Cox proportional hazard models through using the test of proportional assumptions. If outliers were found in any of the models, they were explored to determine the characteristics of the observations that made them outliers and ensure data were not erroneous.

RESULTS

A total of 80 calves were enrolled in the trial with 40 calves randomly assigned to the control, and 40 calves were randomly assigned to the NG group. The mean weight of the calves at arrival was 104 lbs; no statistical differences were found between the treatment groups using a Wilcoxon rank-sum test ($P = 0.32$). The average serum total protein level was 5.79 g/dL with 20% of calves having FPT. The level of serum total protein tended to be lower in the NG group ($P = 0.08$) using a t-test, however, the incidence of FPT was not different between the NG and control group ($P = 0.26$) using a χ^2 test. A total of 21 calves were sourced from a drover, with 14 and 7 calves being in the control and NG groups, respectively. This tended to be different between groups ($P = 0.08$).

Diarrhea

The proportion of time with abnormal fecal scores was calculated by dividing the number of days with an abnormal fecal score by the number of days the calves were fecal scored. Calves spent 19.5% and 14.8% of the first 28 days with a fecal score ≥ 2 in the control and NG groups, respectively ($P = 0.15$). In the generalized linear model, the treatment group, weight at arrival, and level of total

protein were significant. The NG group had a lower proportion of the scoring period with a fecal score ≥ 2 ($P = 0.03$) after controlling for weight at arrival and the level of total protein (**Table 1**).

Calves had a fecal score of 3 for 8.5% and 4.2% of the first 28 days in the control and NG groups, respectively ($P = 0.008$). The NG group had a lower proportion of time with a fecal score of 3 when compared to the control group in the generalized linear model ($P = 0.001$). Weight at arrival and the level of total protein were both associated with the outcome (**Table 2**).

Treatment for Diarrhea

Overall, 53 calves (66.3%) were treated (meloxicam and trimethoprim sulfa) and at least once for diarrhea, with 80% of the control group and 47.5% in the NG group being treated ($P = 0.009$). The NG group had a lower odds of being treated for diarrhea during the growing period in the logistic regression model ($P = 0.02$). The level of total protein measured at arrival was also associated with diarrhea treatment (**Table 3**).

Of those that received initial treatment, 16.9% of the calves in the experiment received further treatment for diarrhea. In the control group, 18.8% of calves that received an initial treatment were treated again, whereas, in the NG group, 14.3% of calves relapsed ($P = 0.67$). A logistic regression model was created; however, no statistical differences were found between treatment groups ($P > 0.05$). Weight at arrival and the level of total protein were associated with a relapse treatment.

Respiratory Disease

A total of 46 calves (57.5%) were treated once for respiratory disease during the experimental period. In the control group, 62.5% of the calves were treated, whereas 52.5% of calves in the NG group were treated. No differences were found with a Chi-square test ($P = 0.37$) and in a logistic regression model (**Table 4**). Of the calves that were treated once, 56% and 43% of calves in the control and NG group were treated again, respectively. No statistical differences (P 0.38) between the groups were found with a Chi-square test.

Growth

The weights at 14, 49, 56, and 78 days following arrival were 111.3 lbs, 172.0 lbs, 182.2 lbs, and 230.8 lbs, respectively, in the control group. The NG group the weights were 111.6 lbs, 179.3 lbs, 205.1 lbs, and 253.2 lbs, respectively. These weights were significantly different by treatment group at day 56 ($P = 0.003$) and 78 ($P = 0.03$) when using a t-test.

Average daily gain (**ADG**) for the periods of 0 to 49 days, 0 to 78 days, and 49 to 78 days were calculated. Average daily gain from 0 to 49 days was 1.37 lbs/day and 1.54 lbs/day for the control and NG groups, respectively. No statistical differences were found with a t-test ($P = 0.11$). A linear mixed model was built to evaluate the 0 to 49-day period and found no significant differences between treatment groups. The weight at arrival and level of total protein were associated with growth in the 0 to 49-day period (**Table 5**).

Average daily gain from 0 to 77 days after arrival was calculated to be 1.61 lbs/day in the control group, whereas the NG group had an ADG of 1.91 lbs/day. Using a Wilcoxon rank sum test, the NG group had a significantly higher ADG ($P = 0.02$) than the control group. In a linear regression model, the NG group had a 0.29 lb/d advantage in growth compared to the control group. Weight at arrival and total protein also had an impact on growth. (**Table 6**).

The gain following weaning (49 to 78 days after arrival) was found to be 1.99 lbs/day and 2.52 lbs/day for the control and NG groups, respectively. There was a tendency ($P = 0.07$) for the ADG to be higher in the NG group using a t-test. The linear model found that the NG group tended to have a 0.57 lb/d increase in ADG in the post-weaning period. Weight at arrival was also associated with ADG between 49 to 78 days following arrival.

Feed Efficiency

Feed efficiency was calculated for the entire experimental period, pre-weaning period, and post-weaning period using the total amount of feed consumed (milk replacer and concentrate) divided by the number of lbs gained. The feed efficiency for the entire experimental period was 3.03 lbs of feed per 1 lb of gain and 2.44 lbs of feed per 1 lb of gain for the control and NG groups, respectively. This difference was not significant using a Wilcoxon rank-sum test ($P = 0.24$). Feed efficiency during the pre-weaning period was 2.39 lbs of feed per 1 lb of gain in the control group and 2.30 lbs of feed per 1 lb of gain for the NG group. This was not different using a Wilcoxon rank-sum test ($P = 0.87$). In the post-weaning period, the feed efficiency was 5.38 lbs of feed per 1 lb of gain for the control group and 3.07 lbs of feed per 1 lb of gain in the NG group. Using a Wilcoxon rank-sum test, the groups were not statistically different ($P = 0.86$) due to the wide variation in post weaning feed efficiency.