Growth of Periruminant Holstein Bull Calves Fed a Fermentation Extract of *Aspergillus oryzae*

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Abstract

A fermentation extract of the fungus *Aspergillus oryzae* can be utilized as a direct fed microbial (DFM). The objective was to determine if dietary inclusion of an extract of *A. oryzae* would improve the growth of Holstein bull calves from birth through 1wk post weaning; it was hypothesized that it would. Calves were randomly assigned to a slaughter age, 4 wk (n = 16) or 8 wk (n = 36) and treatment, control (CON**;** n = 27) or DFM (n = 25). Calves averaged 43.2 ± 1.0 kg of body weight (BW) and 2.8 ± 0.3 days of age at the beginning of the experiment. Calves were housed and fed individually; no bedding was used. Calves assigned to DFM were fed 2 g/d of DFM. Liquid DFM was delivered in milk replacer for the first 4 wk of the trial; solid DFM was top-dressed on texturized grain thereafter. Calves were fed non-medicated milk replacer twice daily (22.0% CP, 20.0% fat DM basis; 680 g/d) and were weaned upon consumption of 0.91 kg of grain (20% CP, 2.0% fat; medicated with decoquinate) for 3 consecutive days or on d 45 of the study, whichever came first. Calves had ad libitum access to grain and water throughout the trial. Feed intake was recorded daily, and BW was recorded weekly. Total dry matter intake per calf did not differ from 0 thru 4 wk (20.3 ± 0.6 kg of DM) or 5 thru 8 wk (58.0 ± 2.08 kg of DM). The gain to feed ratio did not differ by treatment: from 0 thru 4wk (0.55 ± 0.03) or 5 thru 8wk (0.52 ± 0.03). There was no effect of treatment on BW, wither height, or hip height.. Thus, dietary inclusion (2 g/d) of an extract of *A. oryzae* did not result in improved calf growth when supplemented animals were compared to cohorts not fed the DFM. It is possible that the dose used here was not high enough to elicit treatment effects. Given that effects have been noted in other species, a follow-up dose titration study with similar diets as used here seems warranted.

Introduction

The time from birth to weaning is a stressful time for dairy calves. In approximately 8 weeks, dairy calves must not only survive the birthing process, adjust to enteric nutrition, and achieve homeostasis with their extra-uterine environment, they must also change their metabolism from that of a functional monogastric to a functional ruminant (Davis and Drackley, 1998).

Amaferm (Biozyme, Inc.; St. Joseph, MO) is a fermentation extract of *Aspergillus oryzae*; it is a direct fed microbial with prebiotic properties. Amaferm has been shown to increase the natural population of ruminal lactate utilizing bacteria, *Megasphaera elsdenii* (Beharka and Nagaraja, 1998). This may be of benefit to rumen development in young calves because the metabolism of lactate yields butyrate, the most well-known stimulator of rumen development. It is believed that an increase in rumen development will have a positive effect on feed efficiency and growth in cattle. This may also help to lower the stress animals face during the transition period from that of a functional monogastric to a ruminant.

Amaferm has also been shown to have favorable outcomes in fiber digestibility in both ruminants and nonruminants (Chang et al., 1999). However, Amaferm use in dairy calves is relatively less explored. To our knowledge, only one published report involving Amaferm and dairy calves, functional monogastrics, exists (Beharka et al., 1991). In addition, during the trial by Beharka et al. (1991), calves were fed forage in the diet. This practice is outdated in today's industry, creating the need to reevaluate Amaferm and its effects on calves using the "modern" diet with no added forage except that possibly from the texturized starter.

Hypothesis and Objective

The utility of 2 g/d of Amaferm in preweaned calf diets may lie in its inclusion in the milk or milk replacer portion of the diet for the first 4 weeks of life, where intake can be controlled and assured (this may affect lower gastrointestinal tract development; less than 3% of consumed liquid is expected to enter the rumen; Toullec and Guilloteau, 1989). Starting around 4 weeks of age, starter consumption increases and becomes more consistent in dairy calves. Thus by 4 weeks of age, 2 g/d of Amaferm can be added to the starter grain instead of the liquid portion of the diet so that more of it may reach the rumen. Amaferm intake by calves may increase feed efficiencies.

The objective of the trial was to determine if a dietary inclusion of an extract of *Aspergillus oryzae*, used as a direct fed microbial (**DFM**), would improve growth and feed efficiency of Holstein bull claves from birth thru 1 week post weaning.

Materials and Methods

Animals and Treatments

The Ohio State University Animal Care and Use Committee approved all animal procedures (Protocol # 2013A00000059). In this 8 week trial, 52 locally sourced (Wayne County, OH) Holstein bull calves were purchased and transported to the OARDC Small Ruminant Research Center (Wooster, OH) on May 6th, 2013. Upon arrival, calves were blocked by source farm (n = 14), age (0 to 6 d of age), initial body weight (**BW**), initial immunoglobulin G (**IgG**) score (moderate or high), and lactation number of dam (primiparous or multiparous) and randomly assigned to a slaughter age (4 week (n = 16) or 8 week (n = 36)) and treatment (control (**CON;** n = 27) or (DFM; n = 25). Calves were individually housed and fed in a naturally ventilated barn; the experimental unit was calf. Pens had no bedding. Calves assigned to DFM received 2 g/d of liquid Amaferm product, delivered in equal portions of milk replacer (**MR**) for the first 4 weeks of the trial. For the final 4 weeks of the trial, DFM was top-dressed on calf starter (2 g/d; offered at the 600 h feeding).

Data Collection

Calves were fed non-medicated MR (22% CP and 20% fat, DM basis) from buckets twice daily at 600 and 1800 h. Calves were offered 0.68 kg of MR powder mixed in 3.78 L of water per day; MR was delivered in 2 equal portions. Calves had free choice access to a medicated (0.0033% Decoquinate; Alpharma Inc., Bridgewater, NJ) 20% CP (DM basis) calf starter and water at all times. Calves began the 5 day weaning process when they consumed 0.91

kg/d of starter for 3 consecutive days or on day 45 of the trial, whichever came first. All 4 week calves remained on milk until slaughter. During the weaning process, MR amount was reduced by 50% and was offered at the 600 h feeding only (Roth et al., 2009). Fresh grain and MR samples were collected once per week to determine DM. Calf starter refusals were collected daily and composited weekly for DM analysis. For the first 4 wk of the trial, grain refusals from all calves were mixed for sampling. Beginning at wk 5, DFM and CON were sampled separately to account for potential differences caused by addition of the DFM on grain .

To analyze DM, grain samples were placed in an oven set at 50°C for 24 h. Milk replacer powder was placed in an oven set at 100°C for 5 h. Body growth was recorded weekly by recording BW, withers height **(WH),** and hip height **(HH).**

Statistical Analysis of Data

Growth and intake data were analyzed using the MIXED procedure of SAS (2004). Growth data were analyzed using the repeated measure of week and fixed effects of treatment, side of the barn (different floor type), and their interactions. An autoregressive covariance structure [AR(1)] was used. Intake data were analyzed with a model that included the fixed effects of treatment, slaughter age, side of the barn, and their interactions. Calf within treatment, slaughter age, and side of the barn was the random effect for each mixed model.

Side of the barn was included as a fixed model effect in all variance models because there were different flooring types on the east and west sides of the barn (expanded metal and slatted plastic, respectively). It was unknown if flooring would affect experimental measurements. We accounted for this potential source of variation in our statistical models, but do not report side of barn effects here because there were none. It should be noted however that this fixed effect was interacted with all other fixed effects in the model and thus partitioned away from the residual error term in our reported results. Least squares means ± standard error of the means are reported unless otherwise noted. Significance was declared when $P \leq 0.05$.

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Results and Discussion

There were no differences between CON and DFM calves regarding MR, starter, and total dry matter intake (**DMI**) (Table 1). Also, there were no treatment by age interactions for these variables. In addition, gain:feed and age at weaning were similar between the treatment.

Although BW, WH, and HH increased as expected with age, there were no differences between CON and DFM for these growth variables (Figure 1). Average daily gain (**ADG**) throughout the trial also was not affected by treatment (Figure 2).

It is possible that the dose used in the trial was not high enough to elicit treatment effects. The 2 g/d of Amaferm recommendation has no substantial findings to back up the dose listed on the label. Also based on past research (Beharka et al., 1991), had we fed forage in this trial, we may have observed a treatment effect. Amaferm has been shown to have benefits when animals are fed a forage diet. As the industry has moved away from feeding calves forage until after weaning, the trial was designed to address this "modern" practice.

Given that responses with this product have been noted in the bovine and other species, a follow-up dose titration study with various calf diets (similar to this trial and added forage) seems warranted.

Conclusions

Contrary to our hypothesis, feeding 2g/d of Amaferm (DFM) to periruminant Holstein bull calves had no effect on milk replacer, grain, or total DMI, gain to feed ratio, and days to weaning. Body weight, WH, and HH and ADG also did not differ by treatment. Regardless of treatment, all calves grew with time, as expected. Calf growth data reported here are consistent with data from other experiments likewise performed under thermoneutral conditions.

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Bibliography

Beharka, A. A., and T. G. Nagaraja. 1998. Effect of *Aspergillus oryzae* extract alone or in combination with antimicrobial compounds on ruminal bacteria. J. Dairy Sci. 81:1591-1598.

Beharka, A. A., T. G. Nagaraja, and J. L. Morrill. 1991. Performance and ruminal function development of young calves fed diets with *Aspergillus oryzae* fermentation extract. J. Dairy Sci. 74:4326-4336.

Chang J. S., E. M. Harper, and R. E. Calza. 1999. Fermentation extract effects on the morphology and metabolism of the rumen fungus *Neocallimastix frontalis* EB 188. J. Appl. Microbiol. 86:389–398.

Davis, C. L., and J. K. Drackley. 1998. The development, nutrition, and management of the young calf. Iowa State Univ. Press, Ames.

Roth, B. A., N. M. Keil, L. Gygay, and E. Hillmann. 2009. Influence of weaning method on health status and rumen development in dairy calves. J. Dairy Sci. 92:645-656.

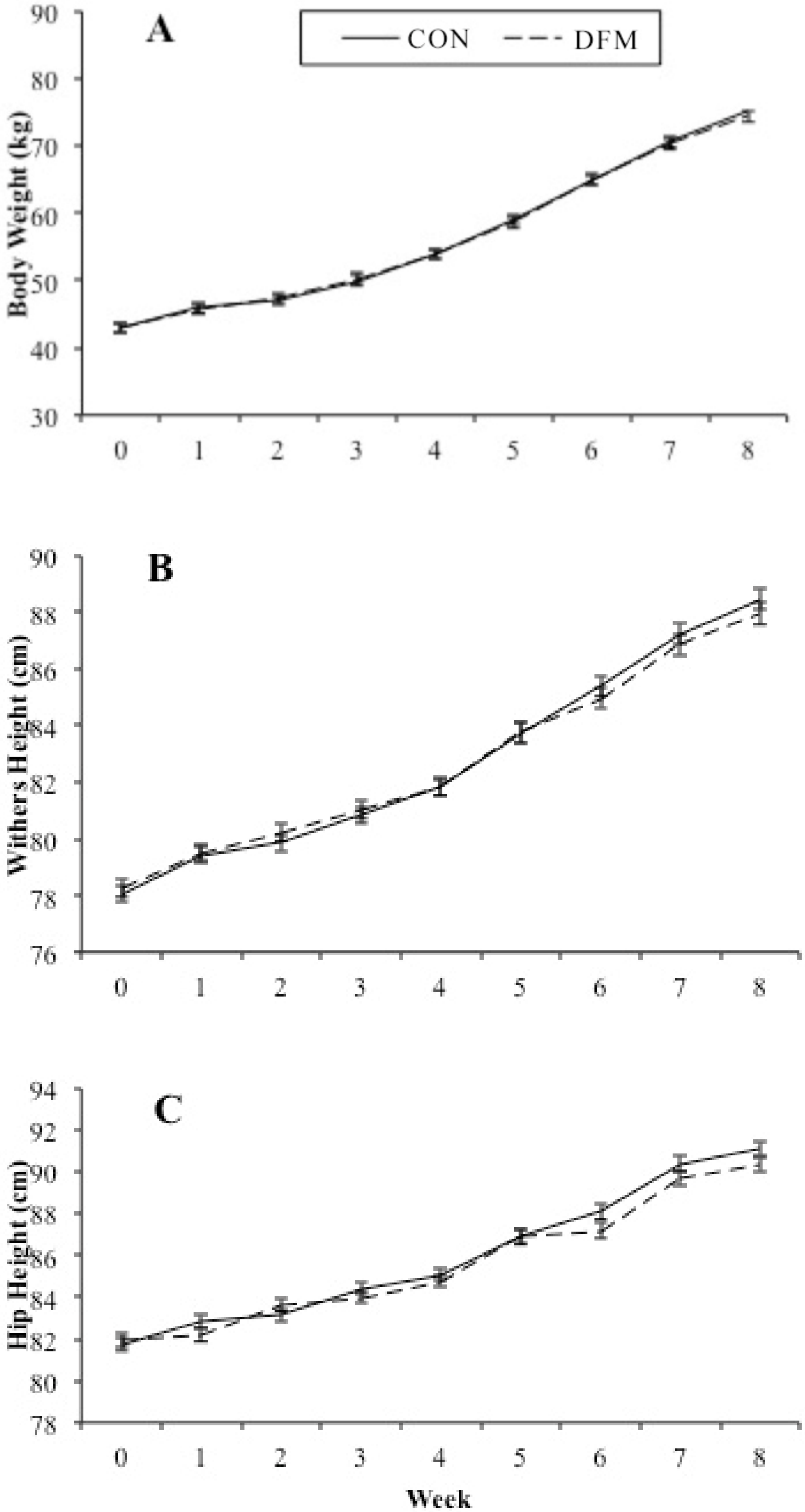
SAS. 2004. SAS/STAT 9.3 User's Guide. SAS Inst., Inc., Cary, NC.

Toullec, R., and P. Guilloteau. 1989. Research into the digestive physiology of the milk-fed calf. In: E. J. van Weerden and J. Huisman (Ed.) Nutrition and Digestive Physiology in Monogastric Farm Animals. p 37. Puduc, Wageningen, The Netherlands.

Table 1. Milk replacer (MR), starter, and total dry matter (DM) intake in Holstein bull calves fed either a control diet or control + fermentation extract of *Aspergillus oryzae* and slaughtered at either 4 or 8 wk of age

	Diet[2]					Test of fixed effects,[4] *P*-value		
	CON		DFM					
Item[1]	4 wk (n = 27)	8 wk (n = 19)	4 wk (n = 25)	8 wk (n = 17)	SEM	Treatment	Age[3]	Treatment x Age
MR intake								
DM, kg	16.5	27.0	16.6	27.2	0.6	0.78	< 0.001	0.90
Starter intake								
DM, kg	3.8	31.0	3.2	32.9	3.1	0.82	< 0.001	0.63
Total intake								
DM, kg	20.3	58.1	19.7	60.2	2.6	0.74	< 0.001	0.55
Gain:Feed	0.55	0.55	0.58	0.52	0.03	0.96	0.20	0.26
Weaning, d[5]	--	40.4	--	40.4	0.8	0.99	--	--

[1]4 wk calves = 52 calves that were alive during the first 4 wk of the trial; 8 wk = 36 calves that remained on the trial after the first slaughter at the 4 wk time point.
[2]Diets: CON = calf starter with 20% CP (DM basis), offered ad libitum daily with no fermentation extract of *A. oryzae* included (n = 27); DFM = calf starter with 20% CP (DM basis), offered ad libitum daily with 2 g/d of fermentation extract of *A. oryzae* included (n = 25). Both CON and DFM diets were fed a 22% CP, 20% fat (DM basis) non-medicated MR.
[3]Age represents 4 or 8 wk slaughter time point. A *P*-value is not reported for age when age was not included in the model.
[4]Significant declared when $P \leq 0.05$.
[5]Number of days until 8 wk slaughter calves (n = 36) began the weaning process.



A
CON
DFM
Body Weight (kg)
B
Withers Height (cm)
C
Hip Height (cm)
Week

Figure 1. Body weight (BW), withers height (WH), and hip height (HH) throughout the duration of the trial were not affected by treatment (*P* = 0.90, *P* = 0.78, and *P* = 0.12, respectively), but all three increased throughout the trial (P < 0.001). From wk 0 to wk 4 of the trial, n = 27 for CON and n = 25 for DFM; whereas, from wk 4 to 8 of the trial, n = 19 for CON and n = 17 for DFM. Treatments: CON = calf starter with 20% CP (DM basis), offered ad libitum daily with no fermentation extract of *Aspergillus oryzae* included (n = 27); DFM = calf starter with 20% CP (DM basis), offered ad libitum daily with 2 g/d of fermentation extract of *A. oryzae* included (n = 25)) in A, B, and C, respectively. Both CON and DFM diets were fed a 22% CP, 20% fat (DM basis) non-medicated MR.

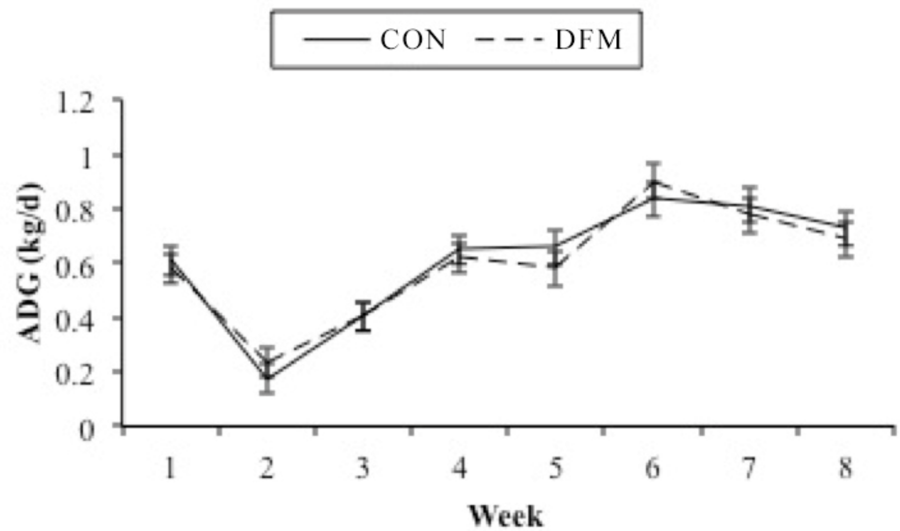


Figure 2. Average daily gain (ADG) throughout the duration of the trial was not affected by treatment (*P* = 0.70) but did increase throughout the trial (*P* < 0.001). From wk 0 to 4 of the trial, n = 27 for CON and n = 25 for DFM; whereas, from wk 4 to 8 of the trial, n = 19 for CON and n = 17 for DFM. Treatments: CON = calf starter with 20% CP (DM basis), offered ad libitum daily with no fermentation extract of *Aspergillus oryzae* included (n = 27); DFM = calf starter with 20% CP (DM basis), offered ad libitum daily with 2 g/d of fermentation extract of *A. oryzae* included (n = 25). Both CON and DFM diets were fed a 22% CP, 20% fat (DM basis) non-medicated MR.