Feedlot Trilogy: Acidosis prevention, cereal grains and grain processing, and liver abscess control strategies

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Introduction

Feedlot production systems in North America historically have been very technologically driven, drawing from a wide range of resources to capture efficiencies of production. An evolving regulatory climate, changes in consumer preferences, and access to new technologies are important factors that impact the business landscape, and thus the need for research. Our research program, as is the case with many others, is thus multi-faceted. This paper provides an overview of several research topics that have been emphasized in our program in the past several years, including utilization of novel types of cereal grains, acidosis prevention with lactic-acid utilizing bacterium, and strategies for decreasing usage of in-feed antibiotics in feedlot cattle.

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A New Stategy for Controlling Acidosis

In modern systems of beef and dairy cattle production, energy-dense feeds such as cereal grains, cereal grain co-products, syrups, whey, and other products often are fed as energy sources to improve growth rate, milk production, and efficiency of feed utilization. The inherently rapid rates of fermentation of these ingredients can result in the overproduction and accumulation of organic acids in unadapted animals, or in adapted animals that overconsume, thereby decreasing ruminal pH. These conditions impede productivity and can compromise growth performance or animal health and well-being, often with severely debilitating or life-threatening outcomes. Feeding diets that are rich in non-structural carbohydrates predisposes ruminants to digestive disturbances, and gradual adaptation to highly fermentable diets and subsequent regularity in feeding practices are regarded as essential in minimizing the occurrence of ruminal acidosis and related maladies, such as bloat, liver abscess, endotoxemia, respiratory disease, and laminitis. Gradually shifting the proportion of rapidly fermentable dietary substrate allows ample time for establishment of key microbial populations, including *Megasphaera elsdenii*, that otherwise are represent only a small fraction of the microbial population.

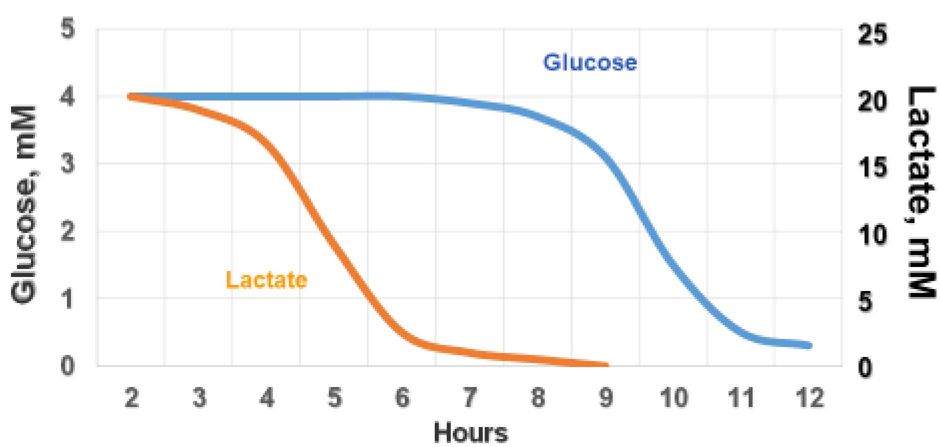
Herein an alternative approach to diet transition is described in which animals are inoculated with live cultures of the probiotic bacterium, *Megasphaera elsdenii* (Lactipro; MS Biotec). Most commonly the transition to concentrates in the absence of exogenous *Megasphaera* is accomplished by feeding a series of 3 to 5 step-up diets for which the roughage component is progressively decreased and replaced by increasing proportions of highly fermentable carbohydrate sources, such as cereal grains or grain byproducts. As animals progress through the diets, the populations of flora and fauna in the rumen shift from those that are adept at digesting cellulosic materials to those that digest non-structural carbohydrates. Key in this process is maintaining a balance between organic acid production and organic acid absorption and utilization, thus avoiding major shifts in ruminal pH. The step-up process most commonly is implemented over a period of three weeks, starting with 40 to 50% roughage and ending with a final finishing diet containing 6-8% roughage. Other step-up strategies have been developed, such as the two-ration feeding system, in which cattle initially are fed 3 meals per day of a diet containing 50-60% concentrate upon arrival in the feedlot. After 6 to 7 days, cattle are then fed the high-roughage diet (i.e., 40-50% roughage) for the first and second feedings, and then fed the finisher diet (6-8% roughage) at the third feeding. This is continued for 6-7 days or more, after which cattle are fed the high-roughage for the first meal, and the finish diet for the second and third meals. This is repeated for 6-7 days or more, after which cattle receive the finish diet at all three feedings, which normally begins on or around day 21 after arrival in the feedlot. Numerous variations of this this method are used, but all attempt to satiate cattle at early feedings using a diet that contains more roughage, followed by higher concentrate levels in subsequent feedings when cattle are less likely to exhibit overeating behaviors. Proportions of concentrate consumed over a 24-hour period are thus changed gradually, just as with the multi-ration step-up system. Regardless of the method used, common to all step-up strategies is the goal of increasing proportions of concentrate gradually to allow for changes in the microbial population, as well as changes in the animals' capacity to absorb and metabolize increasing amounts of fermentative end-products.

Abrupt changes in the proportion of concentrates in finishing cattle diets can have dire consequences for finishing cattle. Opportunistic, highly prolific bacteria, such as *Streptococcus bovis* and *Lactobacillus sp*. colonize the rumen rapidly in the presence of these substrates, metabolizing the carbohydrates to yield substantial amounts of D,L-lactate. *Streptococcus bovis* is regarded as a key microorganism in the manifestation of acidosis, as it metabolizes starches and sugars to produce large amounts of lactic acid as an end-product of its metabolism. Lactic acid is a relatively strong acid (pKa=3.8) in comparison to the major volatile fatty acids (pKa=4.8), and its production by *S.bovis* frequently is associated with the initial decline in ruminal pH (Nagaraja and Titgemeyer, 2007). The resulting decrease in ruminal pH favors growth and activity of other lactate producers, such as *Lactobacillus* sp., further exacerbating the imbalance between lactate production and lactate utilization. The imbalance is greatest during acute bouts of acidosis (Chen, et al, 2019), where ruminal pH can drop below 5 and jeopardize well-being of the animal.

Lactate-utilizing species of microorganisms normally are present in relatively low numbers in forage-fed animals. For example, we measured populations of *Megasphaera elsdenii* in steers fed a diet consisting of alfalfa hay using a quantitative RT-PCR assay (McDaniel, 2009) and determined that baseline populations were approximately 10^4 genomes per mL of ruminal contents, thus comprising a relatively small proportion of the overall bacterial population of 10^{11} cells per gram of ruminal contents reported by Ji et al. (2017). Given the propensity for rapid colonization of the rumen by lactate producers and the relatively small starting populations of lactate utilizers, cattle are very susceptible to imbalance between production and utilization of lactate during the initial stages of feedlot finishing. Excess production of organic acids can alter the rumen environment to favor growth of lactate producers, creating conditions that can spiral into subacute or acute ruminal acidosis. Gradual adaptation to high-concentrate aims to avoid this imbalance by matching organic acid production with absorption metabolism of organic acids, thereby reducing the occurrence of ruminal acidosis.

Megasphaera elsdenii has been identified as the predominant utilizer of lactic acid in grain-adapted animals (Counotte et al., 1981), and is believed to play a central role in maintaining a healthy, productive ruminal environment. A key feature of *Megasphaera elsdenii* is its preferential use of lactic acid as a carbon source, as illustrated in Figure 1. This is key to the success of *Megasphaera* in attenuating ruminal pH depression, as it converts lactate to less potent organic acids, including butyrate, propionate, and acetate, rather than synthesizing organic acids from glucose, as is the case with other organisms. When lactate is depleted the organism immediately begins to metabolize other substrates, thus ensuring its survival under conditions of low lactate availability.

Figure 1. Substrate disappearance from *Megasphaera elsdenii* cultures containing a mixture of glucose and lactate as carbon sources. Lactate is utilized almost entirely before organisms begin to metabolize glucose. Adapted from Hino et al, 1994, Applied and Environmental Microbiology, V60:1827-1831.

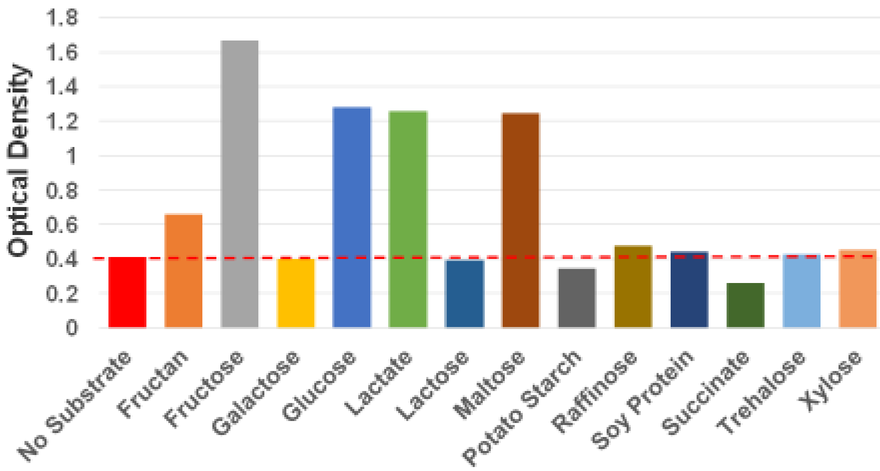


The potential for use of *Megasphaera elsdenii* as a probiotic bacterium was recognized relatively soon after its initial isolation. Its use for prevention of lactic acidosis has been the subject of several patents, the first of which was granted as early as 1976 (Hahn and Abdo, 1976; Das, 1979; Leedle et al., 1995). Early attempts to commercialize strains of *Megasphaera elsdenii* were hindered by several factors, including one or more of the following: insufficient replication rate within industrial fermenters; inhibition by ionophores within the rumen; poor replication at low ruminal pH; failure to preferentially use lactic acid as substrate; or inability to utilize substrates other than lactic acid (Meissner et al., 2010). Variance among isolates with respect to these characteristics is substantial, suggesting it is feasible to overcome these limitations. We examined nearly 300 isolates of *Megasphaera elsdenii* obtained from the ceca of horses fed diets consisting predominantly of roughage or a concentrate-roughage mixture, and observed more than 10-fold differences in growth rate and capacity for lactic acid utilization (unpublished data) among isolates. Moreover, some isolates grew poorly at low pH, and thus were not affective in attenuating lactic acid accumulation, while others had generation intervals of less than 50 minutes and aggressively metabolized lactate.

The first successful commercialization of *Megasphaera elsdenii* did not occur until the mid-2000s with an initial appearance in South Africa, followed several years later by regulatory approval and introduction into the U.S. market in 2010. The commercialized strain, NCIMB 41125 (sold under the trade name, Lactipro), was developed in South Africa, and is the product of a very rigorous selection process aimed at overcoming limitations encountered in previous commercialization attempts. In this process, candidate strains were selected only from grain-fed animals and were isolated under conditions that favored capacity for rapid replication at low pH and in the presence of relatively high concentrations of lactic acid. Consequently, NCIMB 41125 has remarkable capacity for lactic acid utilization and replicates quickly, and thus is well-suited to production in commercial fermenters. Additionally, the strain maintains vibrant growth and metabolic activity in the presence of ionophores, antibiotics, beta agonists, anthelmintics, and other feed additive compounds. Cultures also are substantially aerotolerant, withstanding oxygen presence for up to several hours without appreciable loss of viability. The organism also has the ability to utilize carbon sources other than lactate, which is an important feature if the organism is to persist within the gut in the absence of large quantities of lactic acid. Figure 2 summarizes a laboratory experiment we conducted (Mobiglia et al, 2016) to assess relative growth of NCIMB 41125 when cultivated with different carbon sources. This and other experiments have revealed that the organism grows exceptionally well with maltose, glucose, fructose, and sucrose (not shown) as substrates, and to a lesser extent with fructooligosaccharide (fructan). When presented combinations of substrates, however, the strain preferentially utilizes lactic acid over other carbon sources. These factors are crucially important, recognizing that the objective in using *Megasphaera* as a probiotic is to ensure that a sufficient population of lactate-utilizing bacteria are present *prior* to a dietary challenge. The organism must therefore be capable of colonizing the rumen utilizing substrates other than lactate (i.e., prior to a dietary challenge) in the absence of appreciable amounts of lactate, to ensure its presence is maintained when ruminal metabolism shifts to yield more lactate. As readily fermentable carbohydrates are added to the diet in greater proportions, more lactic acid is generated, and the presence of substantial numbers of

Megasphaera ensures that a metabolic insult is avoided by metabolizing the acid to yield volatile fatty acids.

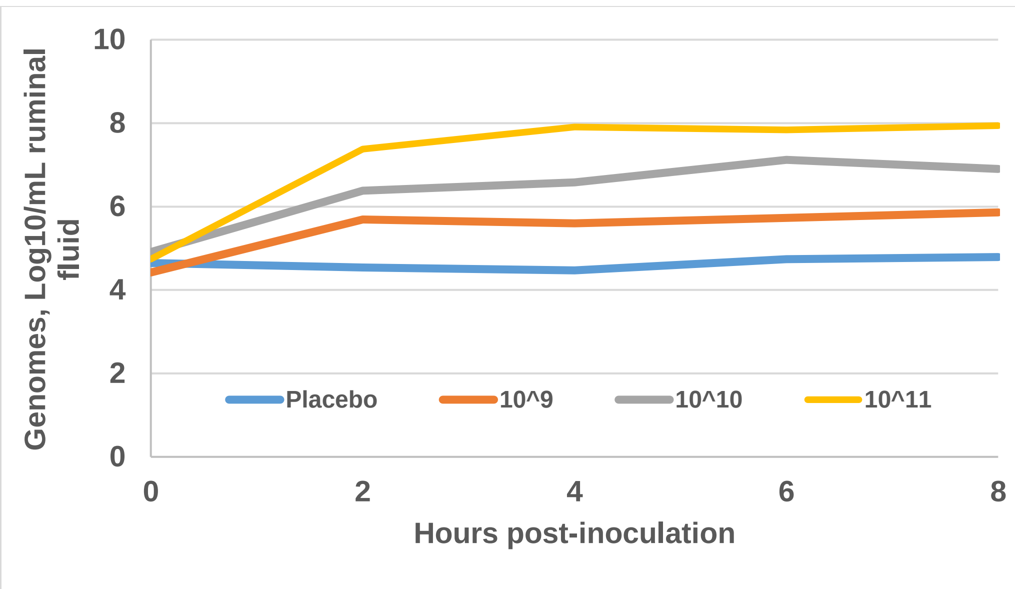
Figure 2. Substrate utilization by *Megasphaera elsdenii*. Cultures were grown on media containing yeast extract, peptone, minerals, vitamins, and the respective carbon source. The assay measures change in optical density after 24 hours. Bars above the dashed red line (i.e., control) are presumed to indicate capacity to utilize the respective substrate. Bars at or below the dashed line indicate no capacity or limited capacity to utilize that substrate. Adapted from Mobiglia et al., 2016.



Acidosis remains as a major challenge for beef and dairy producers that use significant proportions of grains and grain byproducts in cattle diets. Adapting cattle to these diets is both time-consuming and costly. Roughages used during the transition period generally have a high cost per unit of energy. Additionally, they contribute disproportionately to manure accumulation due to their low digestibility. Use of multiple step-up diets also increases complexity of the feeding system, and decrease efficiency of the diet fabrication process, thus adding cost to the production system. Alternative strategies are thus needed to minimize occurrence of acidosis while avoiding unnecessary costs.

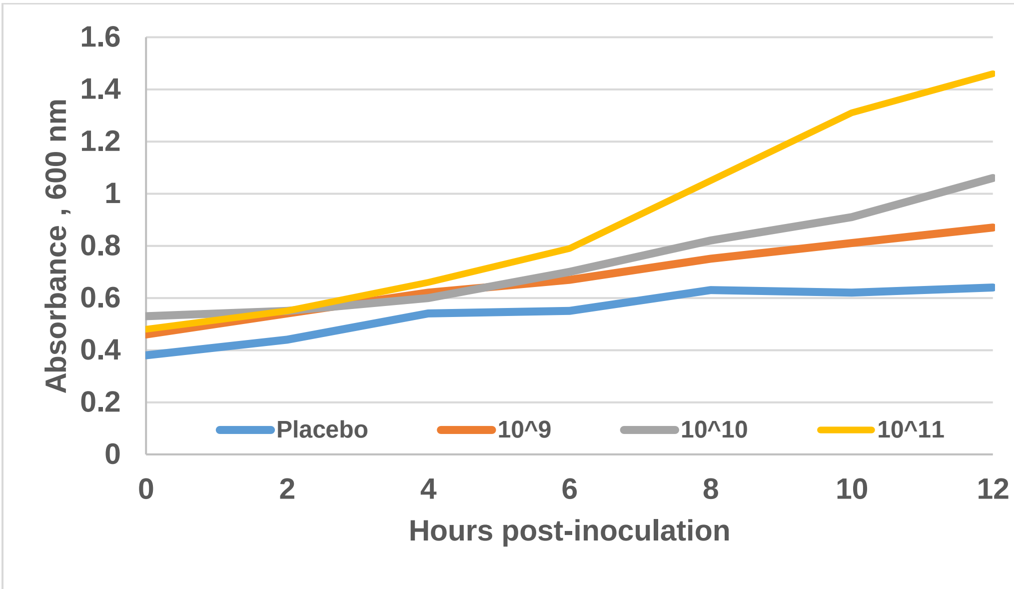
Armed with knowledge of the capacity for *Megasphaera* to utilize lactate preferentially, the objective of our first animal experiments was to determine the dosage (i.e., number of colony forming units) required to prevent acidosis when animals were subjected to a carbohydrate challenge. The key was in finding the minimum inoculation rate that would result in effective ruminal colonization in an effort to contain costs of inoculating animals. Twenty ruminally-cannulated beef steers were used in this experiment (McDaniel, 2009). The cattle were maintained on a diet of alfalfa hay and salt for two weeks. Following the acclimation period, feed and water were withheld for 24 hours, and animals were then dosed with 0, 1 billion, 10 billion, or 100 billion colony-forming units of *Megasphaera elsdenii* via the ruminal cannula. Animals were immediately fed a diet consisting of 65% finely ground wheat grain (a diet know to provoke acidosis). Samples of ruminal contents were obtained via the ruminal cannula before and at several points after inoculation to assess changes in microbial populations and organic acid profiles. *Megasphaera* populations in the rumen were ≤10^5 genomes/mL of ruminal fluid for all treatments prior to inoculation, and lactic acid concentrations were undetectable. Figure 3 illustrates changes in populations of *Megasphaera elsdenii* after inoculation and introduction of the concentrate-based diet. Ruminal concentrations of *Megasphaera* in the control group remained below 5 logs 8 hours after inoculation, while inoculated cattle achieved concentrations of 6, 7, and 8 logs per mL of ruminal contents, thus increasing in a dose dependent manner.

Figure 3. Ruminal proliferation of *Megasphaera elsdenii* following inoculation with 0, 1 billion, 10 billion, or 100 billion colony-forming units of a liquid culture (Lactipro). Treatment by time interaction, P < 0.05; Linear effect of dosage size, P < 0.05. Placebo versus *Megasphaera* groups, P < 0.01. SEM = 0.44. Adapted from McDaniel, 2009.



To assess changes in capacity of ruminal flora to metabolize lactic acid, we extracted ruminal contents from steers 8 hours after inoculation and transferred a small amount (1% inoculation rate) into test tubes containing medium with lactate as the predominant carbon source. Changes in absorbance (600 nm) were used as an indicator of bacterial proliferation in lactate medium. Tubes were incubated at 39C for 12 hours, and changes in absorbance were measured at 2-hour intervals post-inoculation. The results of this assay, shown in Figure 3, illustrate that growth of cultures on lactate medium was minimal for tubes inoculated with ruminal fluid extracted from control steers, but increased in a dose-dependent manner for ruminal contents of steers inoculated with 1, 10, and 100 billion colony-forming units of *Megasphaera elsdenii*.

Figure 4. Microbial growth (estimated as changes in optical density) in lactate media inoculated with ruminal contents extracted from steers dosed with 0, 1 billion, 10 billion, or 100 billion colony forming units of *Megasphaera elsdenii.* Treatment by time interaction, P < 0.05; Linear effect of dosage size, P < 0.05. Placebo versus *Megasphaera* groups, P < 0.01. SEM = 0.04. Adapted from McDaniel, 2009.



More direct evidence of changes in capacity for lactate metabolism are shown in Table 1, which summarizes ruminal concentrations of lactate and major VFAs eight hours after steers were inoculated with 0, 1, 10, or 100 billion CFUs of *Megasphaera*. These results reveal a partial amelioration of lactate accumulation with the lowest dosing rate (1 billion CFU), as lactate concentration 8 hours post-inoculation was essentially half that of controls. Administering 10 or 100 billion CFUs of *Megasphaera* resulted in near complete metabolism of lactate. Moreover, acetate and butyrate increased linearly with increasing dosage of *Megasphaera*. Based on results of this and similar experiments, our subsequent studies have used a targeted dosage of 10 billion colony forming units, as this appears to provide sufficient protection against lactate accumulation within the rumen.

Table 1. Ruminal organic acid concentrations 24 hours post-challenge for steers dosed with 0, 1 billion, 10 billion, or 100 billion colony forming units of *Megasphaera elsdenii*. Adapted from McDaniel, 2009.

	Dosage size, CFU/animal					
Concentration, mM	**0**	**10^9**	**10^{10}**	**10^{11}**	**SEM**	**Contrasts[a]**
Acetate	29.3	34.0	32.6	40.3	3.91	L, Q
Propionate	17.4	17.1	28.1	19.7	2.39	-
Butyrate	13.3	9.6	16.0	19.3	2.99	L, Q
Lactate	49.8	24.6	3.5	3.0	4.47	M, L

[a]Significant at $P < 0.05$.
M = Placebos versus average of *Megasphaera* treatments
L = Linear effect of dose size
Q = Quadratic effect of dose size

Feedlot cattle normally are managed in ways that are designed to minimize the occurrence of acidosis. Consequently, probiotic strategies aimed at alleviating acidosis logically would be efficacious only for those animals in the population that are more vulnerable to acidosis. It is feasible, however, to modify feeding systems to more fully exploit the benefits of *Megasphaera*. This was the goal of one of our very early performance studies conducted in 2003. Yearling steers were transitioned to diets containing 94% concentrate and 6% roughage in either 8 or 17 days. The 17-day transition regimen used four step-up diets containing 55, 65, 75, and 85% concentrate, with each diet being fed for 4 days, followed by the finishing diet with 94% concentrate, which was introduced on day 17. This normally would be regarded as an aggressive step-up strategy for USA feedlots, as transition programs are more commonly 21 days or more in duration. We also included a far more aggressive step-up regimen in which cattle were fed the diet containing 55% concentrate for three days and the diet with 75% concentrate for four days, followed by introduction of the 94% concentrate finishing diet on day 8. Cattle within each group received 0 or 10 billion colony-forming units of *Megasphaera elsdenii* as a fresh liquid culture prior to initiating the step-up program. Results of the study are shown in Table 2. Improvements in performance associated with *Megasphaera* administration were relatively small when included as part of the les aggressive (17-day) step-up regimen. The *Megasphaera* cattle gained slightly more, and were moderately more efficient. The aggressive, 8-day transition regimen clearly overwhelmed the control cattle, whereas those that received *Megasphaera* were able to cope with the rapid transition to concentrates, yielding carcass weights comparable to those of control cattle fed the more traditional step-up regimen. This strategy has gained favor among nutritionists and feedlot operators in the USA, South Africa, and Australia, as it: 1) provides for modest improvements in animal efficiency and carcass quality; 2) decreases complexity of the feed milling operation by reducing number of diets that must be fed; 3) decreases manure production as a result of decreased consumption of poorly digested feedstuffs.

Table 2. Feedlot performance and carcass traits of steers transitioned to 94% concentrate diets using a 17-day program with 4 step-up diets each fed 4 days; or an 8-day regimen (3 diets) with or without oral dosing of *Megasphaera*

	17-day step-up		*8-day step-up*			
Item	**Control**	**Mega**	**Control**	***Mega***	**SEM**	**P-value[1]**
Dry matter intake, kg/day[a]	7.87	8.19	7.66	7.64	0.22	0.25
Feed intake variation[b]	117	102	119	84	14	--
Average daily gain, kg	1.49	1.52	1.34	1.48	0.07	0.20
Carcass-adjusted ADG, kg	1.25	1.31	1.08	1.25	0.07	0.09
Liver abscesses, %	4.7	5.5	20.1	9.7	6.6	0.47
Carcass weight, kg	334	338	324	335	9.7	0.10

[1]Main effect of *Megasphaera elsdenii*. [a] Feed intake measured daily for individual animals over the initial 28 days on feed. [b]Coefficient of variation for changes in feed intake during the first 3 days on feed.

In a follow-up experiment we further evaluated this concept, but to greater extreme. Our control program consisted of 3 step-up diets, each fed for 6 days, with introduction of a finishing diet (10% corn silage as the roughage source) on day 19. The *Megasphaera* treatment consisted of a single oral dose of approximately 1 billion CFU of *Megasphaera elsdenii*, and direct placement of cattle onto the finishing diet with no step-up program. Fecal output was measured for the first 24 days on feed to assess impact of feeding less forage. Results of the study are shown in Table 3. Cattle placed directly onto the finishing diet with *Megasphaera* consumed 40 kg less roughage per animal for the 115-day feeding period, and fecal output during the first 24 days alone was decreased by approximately 16 kg of dry matter per animal. Cattle in the *Megasphaera* treatment displayed no clinical symptoms of acidosis in spite of being placed directly onto the finishing diet. Additionally, gain of the *Megasphaera* cattle was similar to that of controls, but feed intake was marginally less and feed efficiency tended to improve (~1.4% improvement relative to the control group). Liver abscess rates did not differ between treatments, suggesting that the occurrence of acidosis was no greater in the *Megasphaera* group than in the control group in spite of the abrupt diet change. Finally, as has been noted in many experiments with *Megasphaera* administration, cattle that received *Megasphaera* at initial processing tended to have improved marbling, resulting in an increase in the percentage of carcasses that received market premiums for high quality grades. The modest improvements in quality grade and efficiency are overshadowed by the logistic benefits associated with a greatly simplified feeding management system, however.

Table 3. Performance and health of high-risk calves[a] orally drenched with *Megasphaera elsdenii* at initial processing.

Item	**Control**	***Megasphaera***	**SEM**	**P-value**
No. of cattle	221	222	--	--
Days on feed	115	115	--	--
Initial weight, kg	402	399	2.44	0.12
Dry matter intake, kg	12.8	12.6	0.12	0.07
Silage DM intake, kg/steer	176.3	146.2	1.34	< 0.01
Fecal output, kg/d	2.3	1.7	0.06	< 0.01
ADG, kg	2.26	2.25	0.034	0.65
Feed:gain	5.68	5.60	0.051	0.14
Liver abscess, %	11.8	10.8	2.14	0.75
Choice + Prime	81.5	87.0	2.54	0.07

Accelerated studies have since been completed in commercial feedyards with thousands of animals, and these studies have yielded benefits comparable or superior to those shown in our experiments. Table 4 summarizes results of a feedlot study with 4,950 yearling cattle with average initial body weight of 372 kg. Cattle were assigned to one of three treatments: A **Control**, consisting of a conventional program of four step-up diets fed for 5 days each, with the finishing diet introduced on day 21; an **Accelerated** treatment for which cattle were dosed with *Megasphaera*, fed the step 3 diet for 5 days, then placed onto the finishing diet on day 6; and the **Direct Finish** treatment for which cattle were dosed with *Megasphaera* at initial processing and placed directly onto the finish diet on day 1. Cattle in the Accelerated and Direct Finish treatments gained more weight but ate similar amounts of feed compared to controls, and thus were approximately 4% more efficient than cattle fed the conventional regimen. Carcass value increased by $9-12/animal for the *Megasphaera* groups as well. According to the proprietors, logistical efficiencies associated with diet manufacturing, feed delivery, and manure removal were of even greater magnitude, allowing them to reduce staff hours and the number of feeding vehicles.

Table 4. Feedlot performance and carcass traits of commercial feedlot steers (4,950 head) transitioned to high-concentrate diets using a conventional 21-day program with 4 step-up diets; an accelerated regimen with *Megasphaera* followed by 5 days on the step 3 diet and then placed onto the finish diet; or a *Megasphaera* treatment for which cattle were placed directly onto the finish diet with no step-up diets. Sixteen replicates were fed for an average of 142 days.

Item	Control	Accelerated	*Direct finish*	SEM	P-value
Initial weight, kg	372	372	371	7.3	0.75
Average daily gain, kg	1.55^{a}	1.59^{ab}	1.61^{b}	0.090	0.07
Dry matter intake, kg/day	9.58^{x}	9.48^{y}	9.53^{xy}	0.367	0.19
Feed:gain	6.24^{a}	6.01^{b}	5.97^{b}	0.132	<0.01
Carcass weight, kg	385.1	387.4	386.9	5.76	0.21
Carcass value, $	1,589	1,598	1,601	20.85	0.05

[a]Crossbred calves (504 bulls, 141 steers; initial body weight = 221 ± 4.9 kg) were received from Texas over a 2-week period in January (2 loads per day; on the 14th, 19th, and 26th).

In U.S. feedlot production systems, approximately 40% of cattle enter feedlots as weaned calves, with the remaining 60% being fed as yearlings. Many of these calves originate in the Southeast U.S., and are transported 1,000 to 2,000 kilometers or more to feedlot destinations in the Central and Southern Plains. Throughout this process the calves are subjected to a wide range of stressors, including separation from dams and herd mates, feed and water deprivation, commingling with unfamiliar animals, exposure to novel pathogens, dramatic climate changes, and transportation over long distances. Cattle subjected to the conditions frequently are viewed as being at “high-risk” for development of bovine respiratory disease (BRD). We have studied BRD for a couple of decades, and have come to the conclusion that symptoms of BRD are not readily distinguishable from symptoms of acute acidosis. Moreover, response to antibiotic therapy often is poor, leading to multiple therapeutic treatments at significant cost to producers. Similarities in clinical symptoms and treatment response failures led us to hypothesize that some of the cattle might actually be afflicted with acute acidosis rather than BRD. We completed two studies to evaluate this hypothesis, once of which is summarized Table 5 (Miller, 2013). High-risk calves were purchased from the Southeastern U.S. and transported to Manhattan, Kansas. At initial processing half of the cattle received an oral drench consisting of 1 billion colony-forming units of *Megasphaera elsdenii*. Cattle were placed into pens a fed the same receiving diet consisting of 45% corn silage and 55% concentrate. Calves dosed with *Megasphaera* ate 6.6% more feed, gained 24% more weight per day, and were 15% more efficient than their counterparts in the control group. Moreover, morbidity rate decreased by 30% and death loss decreased from 4.9% to 3.8%.

Table 5. Performance and health of high-risk calves[a] orally drenched with *Megasphaera elsdenii* at initial processing.

Item	Control	*Megasphaera*	SEM	P-value
Initial weight, kg	200	203	4.9	0.23
Average daily gain, kg	0.644	0.798	0.073	0.02
Dry matter intake, kg/day	4.32	4.61	0.167	0.01
Feed:gain	6.80	5.75	0.59	0.05
Morbidity, % of population				
Total morbidity	37.7	26.4	4.81	0.02
1st antibiotic therapy	32.0	22.0	4.13	0.02
2nd antibiotic therapy	17.4	11.5	2.09	0.03
3rd antibiotic therapy	5.9	4.4	1.22	0.36
Mortality, %	4.9	3.8	1.13	0.50
Medication cost, $/head	19.70	17.06	0.98	0.01

[a]Crossbred calves (504 bulls, 141 steers; initial body weight = 221 ± 4.9 kg) were received from Texas over a 2-week period in January (2 loads per day; on the 14th, 19th, and 26th).

Cereal Grains and Grain Processing

Cereals grains typically represent a major proportion of finishing cattle diets, and optimizing digestion of grains thus is key to maximizing profitability of feedlot operations. Grinding, rolling, high-moisture ensiling, and steam flaking all are common practices employed by feeders to enhance digestibility of grains. These processes vary markedly with respect to capital investments, operating costs, and expected improvements in efficiency of feed utilization. Cereal grain hybrids vary markedly with respect to endosperm structure or other attributes, and it is becoming increasingly evident that these factors can have important implications for cattle performance. Our research efforts on this front have focused mostly on use of corn hybrids that have been genetically modified for high expression of amylase, or through screening of sorghum hybrids characterized by a wide range of genetic diversity.

Enogen® Feed Corn

Enogen® Feed Corn (Syngenta Crop Protection; hereafter referred to as Enogen or EFC) was developed through genetic modification to produce grains containing relatively high concentrations of amylase, an enzyme that plays a key role in digestion of starch. Initially developed for use by the fuel ethanol industry, incorporating as little as 10-15% Enogen into fermenters made it feasible to eliminate the need for use of exogenous amylases. More recently, the focus has shifted to use of Enogen Feed Corn in livestock and poultry production. The potential value of a high-amylase hybrid in feedlot production seemed reasonable to us, recognizing that capacity for secretion of pancreatic amylase is limited in cattle. In ruminants, total tract digestion of starch relies heavily on extensive digestion within the rumen. This is readily evident in the comparison of cattle fed grains processed by dry rolling, high-moisture ensiling, and steam flaking, whereby the proportions of starch appearing in feces decrease progressively with more rigorous methods of grain processing. Our first investigation into the use of Enogen Feed Corn was with steam flaking, in which we evaluated flaking characteristics of Enogen compared to a mill-run variety of corn. In our initial attempts to flake Enogen grain we utilized our standardized processing conditions, which include final moisture content of 21%, steam conditioning time of 40 minutes, and final bulk density of 28 lb/bu (360 g/L). Enogen grain rapidly imbibed moisture, leading to swelling and bridging in the steam chest. Enogen also was characterized by extensive starch hydrolysis, as indicated by high starch availabilities and a strong aroma of maltose. In subsequent attempts we decreased conditioning time and increased bulk density to 30.5 lb/bu (393 g/L), effectively increasing throughput from 6 tons per hour to 9 tons per hour. In vitro gas production, which we regard as an excellent indicator of ruminal starch availability, is shown in Figure 3 for commodity grain flaked to a density of 28 lb/bu and Enogen flaked to a density of 30.5 lb/bu. In spite of being processed to a lesser degree, Enogen maintained a greater degree of gelatinization.

In the process of steam flaking, moisture often is added to grain and allowed to temper prior to steam conditioning. This provides means for maintaining consistent moisture concentrations in the final flaked grain, but also facilitates starch gelatinization. Targeted moisture concentrations in the final flaked grain products vary among feedyards, but typically range from 19 to 23%. Steam conditioning times also vary, and are a key determinant of mill throughput, as steam chests are of fixed volume. We evaluated impact of tempering moisture (0, 3, or 6% added water) and steam conditioning times (15, 30, or 45 minutes) across a range of mixtures of Enogen and commodity corn (0, 25, 50, 75, or 100% Enogen as proportion of mixture). There were no interactions between moisture level and grain type, or between conditioning time and grain type. The amount of moisture added during the tempering process increased starch availability linearly (47.2, 49.5, and 51.2 for 0, 3, and 6%, respectively; $P < 0.01$), and tended to improve 14-hour in situ dry matter disappearance of flaked grains (40.3, 40.7, and 42.0 for 0, 3, and 6% added moisture; $P = 0.06$). Not surprisingly, presence of moisture is a key factor in gelatinization of starch. With respect to tempering times, there also were no interactions. Table 6 summarizes effects of steam conditioning times on starch availability, in situ dry matter disappearance, in vitro gas production, and production of volatile fatty acids during in vitro incubations of flaked grains. The results clearly demonstrate a quadratic response to conditioning time, suggesting that grains can be underconditioned or overconditioned. We have observed similar effects in other studies, and previously have noted that availability of both starch and protein decrease when grains are overconditioned, which may be due to formation of early stage Maillard reaction products that are poorly digested. Conditioning times in our study likely cannot be extrapolated directly to commercial flaking systems, nevertheless the potential for overconditioning exists within any system, and given the increased processing costs associated with extended conditioning times, there is financial incentive to identify optimum conditioning times for grains.

Figure 3. Time course for in vitro digestion of steam-flaked grains. Mill-run corn was flaked to a density of 360 g/L (28 lb/bu) and Enogen was flaked to a density of 393 g/L (30.5 lb/bu). Grain type by time interaction, P < 0.0001; Effect of grain type, P <0.001.

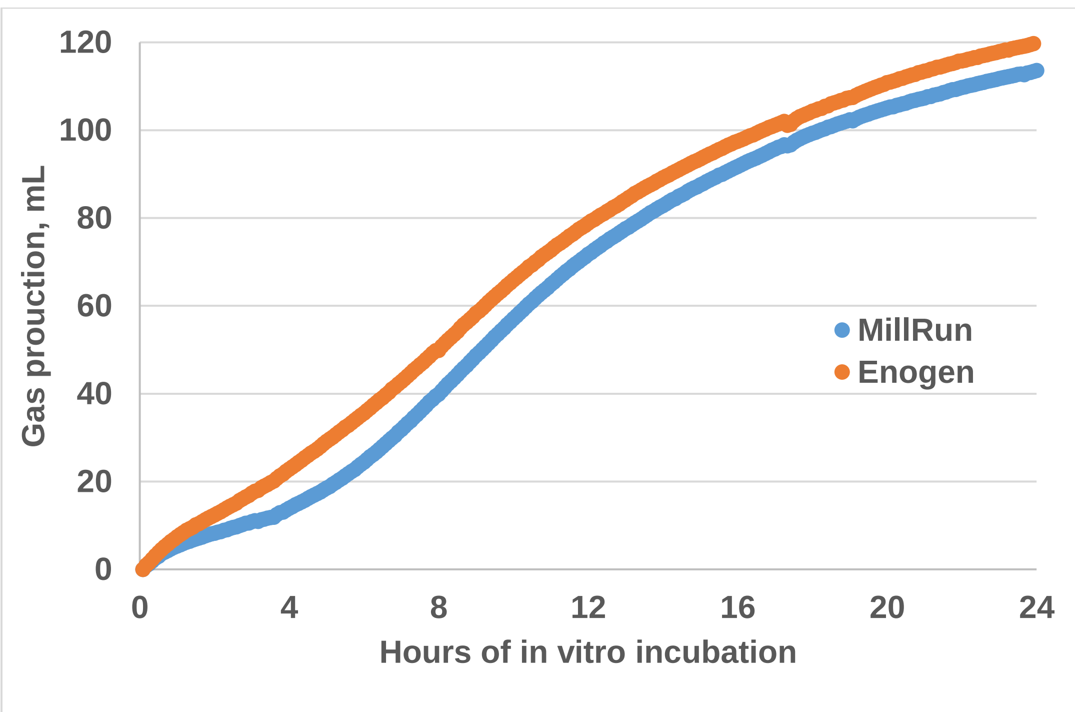


Table 6. Effects of steam conditioning time for flaked Enogen Feed Corn on starch availability, *in situ* dry matter disappearance (ISDMD), and production of fermentative gasses by *in vitro* cultures of mixed ruminal microbes (IVGP)

	Conditioning time, min				*P*-value	
Item	15	30	45	SEM	Linear	Quadratic
Starch availability[1], %	48.5	51.5	47.9	0.60	0.52	< 0.01
ISDMD[2], %	36.6	44.4	42.0	2.66	0.15	0.12
IVGP[3], mL	102.3	121.3	104.9	7.33	0.57	< 0.01
Total VFA[4], mmoles	3.88	4.32	3.78	0.082	0.25	<0.01

[1]Measured using refractive index method (Sindt et al., 2006b) once per sample (90 observations, 30/conditioning time) shortly after flaking.
[2]Measured over 3 d in triplicate by incubating 2.5 g (dry matter; DM) corn flakes in Dacron bags ruminally for 14-h (270 observations, 90/treatment).
[3]Mean gas production during 24-h *in vitro* incubation period. Measured by incubating 3 g (DM) flaked corn as substrate, with 10 mL ruminal fluid inoculum, and 140 mL McDougall's Buffer at 39ºC. Repeated with 2 replicates per sample (90 samples, 180 observations, 60/treatment).
[4]Volatile fatty acids are expressed as mmoles produced per gram of substrate dry matter and were measured by gas chromatography following 24-h incubation of cultures containing 3 g (DM) flaked corn as substrate, 10 mL ruminal fluid inoculum, and 140 mL McDougall's Buffer at 39ºC. Initial incubation was repeated with 2 replicates per sample (90 samples, 180 observations, 60 per conditioning time).

As previously mentioned, the development of Enogen grain targeted the fuel ethanol industry, as the production of ethanol from starch requires conversion of starch to glucose by starch degrading enzymes. Replacement of exogenous enzymes could be accomplished by incorporating as little as 10 to 15% Enogen grain into the process. Consequently, one of our objectives was to determine if Enogen could be used in combination with other grains to enhance digestion of starch from all sources in the diet. Grains were blended, flaked to a density of 28 lb/bu (360 g/L), and then subjected to *in vitro* and *in situ* evaluations. The results, shown in Table 7, indicate that increases in starch availability and *in situ* disappearance were in direct proportion to changes in Enogen content with no indications of a plateau effect. We interpret these observations to suggest that effects of the enzymes are localized, and that there is little migration of enzyme from one grain to the other. It is conceivable that results *in vivo* could be different due to mastication, extensive hydration, and commingling of grains within the rumen, which may more closely emulate conditions in an ethanol fermenter that consists of finely ground grains in a mash form.

Table 7. Effects of increasing proportion of steam-flaked high-amylase corn (EFC) in grain mixtures on starch availability and *in situ* dry matter disappearance (ISDMD)

	Proportion of grain mixture as EFC, %						*P*-value	
Item	0	25	50	75	100	SEM	Linear	Quadratic
Starch availability[1], %	45.0	47.7	50.5	50.7	52.7	0.77	< 0.01	0.16
ISDMD[2], %	34.8	39.5	40.8	43.8	46.0	1.70	< 0.01	0.27

[1]Measured using refractive index method (Sindt et al., 2006b) once per sample (90 observations, 18/treatment) shortly after flaking.
[2]Measured over 3 d in triplicate by incubating 2.5 g (dry matter) corn flakes in Dacron bags ruminally for 14-h (270 observations, 54/treatment).

In vitro and *in situ* evaluations are very useful as screening tools, and essentially allowed us to identify logical targets for processing of Enogen in a feeding study. We followed these experiments with a cattle finishing study involving 700 crossbred beef heifers. Cattle were fed finishing diets with 7% alfalfa hay as roughage, 33 g/ton monensin, no tylosin, and 85.4% flaked corn, either as a commodity grain of unknown hybrid (i.e., mill run) or as Enogen Feed Corn. Mill run corn was flaked to a density of 28 lb/bushel (360 g/L), and Enogen was flaked to a density of 30.5 lb/bu (393 g/L), providing grain throughput of 6 tones per hour for mill-run corn and 9 tones per hour for Enogen. Cattle were fed for a total of 136 days. Dry matter intake did not differ between groups, as presented in Table 8, but gain, final live weight, feed efficiency, and carcass weight all were improved for cattle fed the Enogen-based diet ($P < 0.01$). Interestingly, liver abscesses were less prevalent in heifers fed Enogen (Table 9). Total tract digestibility of starch in cattle fed flaked grains typically is 99% or more, leading us to question the mechanism by which the cattle achieved a 5% improvement in feed efficiency. *In vitro* experiments indicate increases in propionate in proportion to acetate, which is energetically more favorable. We recently have initiated a study to determine site and extent of digestion, as well as fermentative end products, including volatile fatty acids, lactate, and methane. It is our hope that these data will provide insight relative to the underlying mechanism of efficiency improvements with Enogen corn.

Table 8. Feedlot performance and carcass characteristics of heifers fed diets containing steam-flaked mill-run corn (CON) or steam-flaked high-amylase corn (EFC)[1]

Item	CON	EFC	SEM	*P*-value
Initial body weight, kg	395	394	8.6	0.52
Final body weight, kg	588	599	10.7	< 0.01
Dry matter intake, kg/d	10.00	10.07	0.196	0.78
Average daily gain, kg	1.60	1.69	0.028	< 0.01
Feed:gain	6.25	5.95	0.074	< 0.01
Hot carcass weight, kg	366	372	6.41	< 0.01
Longissimus muscle area, cm^2	94.7	94.6	1.02	0.89
12th rib fat thickness, cm	1.16	1.19	0.045	0.21
Marbling score[†]	605	589	10	0.04
USDA Prime, %	6.6	4.9	1.68	0.33
USDA Choice, %	68.7	70.4	4.44	0.62
USDA Select, %	10.7	11.4	2.58	0.79
USDA sub-Select[2], %	9.0	9.3	2.61	0.68
USDA Yield Grade	2.07	2.15	0.069	0.13
Yield Grade 1, %	23.2	19.5	3.77	0.22
Yield Grade 2, %	49.4	47.7	3.01	0.64
Yield Grade 3, %	25.1	30.9	3.07	0.09
Yield Grade 4, %	2.0	2.0	0.76	1.00
Yield Grade 5, %	0.3	0.0	0.20	0.32

[†]500 to 599 = Small degree of marbling; 600 to 699 = Modest degree of marbling.

[1]Trial utilized 700 beef heifers in a randomized complete block design, with 25 animals/pen, 14 pens/treatment, and fed 136 d prior to transport to a commercial abattoir wherein carcass data were collected.

[2]Carcasses graded as USDA Standard, Commercial, Utility, or Cutter.

Table 9. Liver abscess prevalence and severity[1] in heifers fed diets containing steam-flaked mill-run corn (CON) or steam-flaked high-amylase corn (EFC)[2]

Item	CON	EFC	SEM	*P*-value
Total liver abscesses, %	34.4	26.6	2.47	0.03
Mild, %	11.9	12.7	1.80	0.73
Moderate, %	14.7	9.2	1.74	0.03
Severe, %	7.5	4.6	1.40	0.11

[1]Severity measured using Elanco scoring system (Liver Abscess Technical Information AI 6288; Elanco Animal Health, Greenfield, IN). [2]Trial utilized 700 beef heifers in a randomized complete block design, with 25 animals/pen, 14 pens/treatment, and fed 136 d prior to transport to a commercial abattoir wherein livers from each carcass were scored.

Identification of Sorghum Cultivars for Cattle Feeding

Water scarcity in the High Plains cattle feeding region is of growing concern, and efforts to decrease water consumption are deemed essential as a means of preserving the long-term economical livelihood of agricultural-based industries in this region. Kansas is the country's leading producer of grain sorghum, a cereal that is well-adapted to production in arid climates. Nevertheless, relatively little sorghum is used in commercial feedlots, as it is perceived to be nutritionally inferior to corn, and also is more costly to process. We have embarked on a long-term project to evaluate a broad range of sorghum cultivars—both parent lines and hybrids, in an attempt to identify cultivars that can be competitive with corn as energy sources for cattle. *In vitro* screening of more than 2 dozen sorghum cultivars was utilized to identify grains with increased susceptibility to digestion by ruminal microbes, as we regard ruminal disappearance as being the most important contributor to total tract starch digestion. Cultivars with superior *in vitro* digestion characteristics that could be sourced in ample quantities were then used in a feeding study.

For our in vitro screening experiments grains were ground through a 1-mm screen and 3grams of each grain were added to culture flasks containing 125 mL of buffer and 25 mL of strained ruminal fluid. Bottles were capped with an Ankom RF gas pressure monitoring apparatus, and incubated under continuous agitation at 39 Celsius. In vitro gas production, dry matter disappearance, and VFA profiles were used as indicators of susceptibility of grains to microbial digestion. In vitro gas production by microbial cultures in two experiments are summarized in Figures 4 and 5. Sorghum cultivars encompassed a broad range of *in vitro* digestion, as indicated by production of fermentative gasses, some of which were far less than that of the corn control that was used as a benchmark, while others were substantially greater than corn. Profiles of volatile fatty acids (data not shown) also revealed broad divergence among cultivars, with a range of 0.64 to 1.69 for acetate:propionate ratio.

Figure 4. Gas production (experiment 1) by *in vitro* cultures using ground grains as substrate and ruminal fluid as microbial inoculum. SEM = 1.2. Effect of cultivar, P < 0.0001.

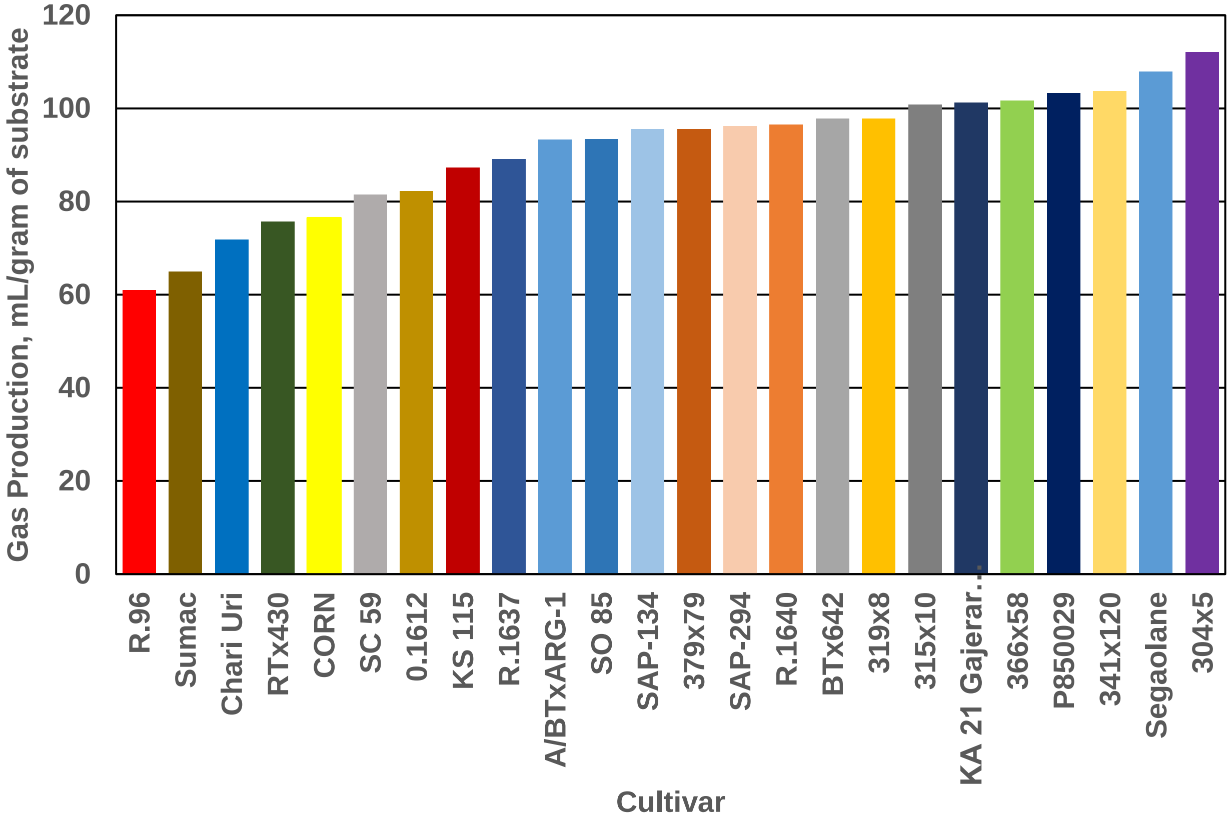


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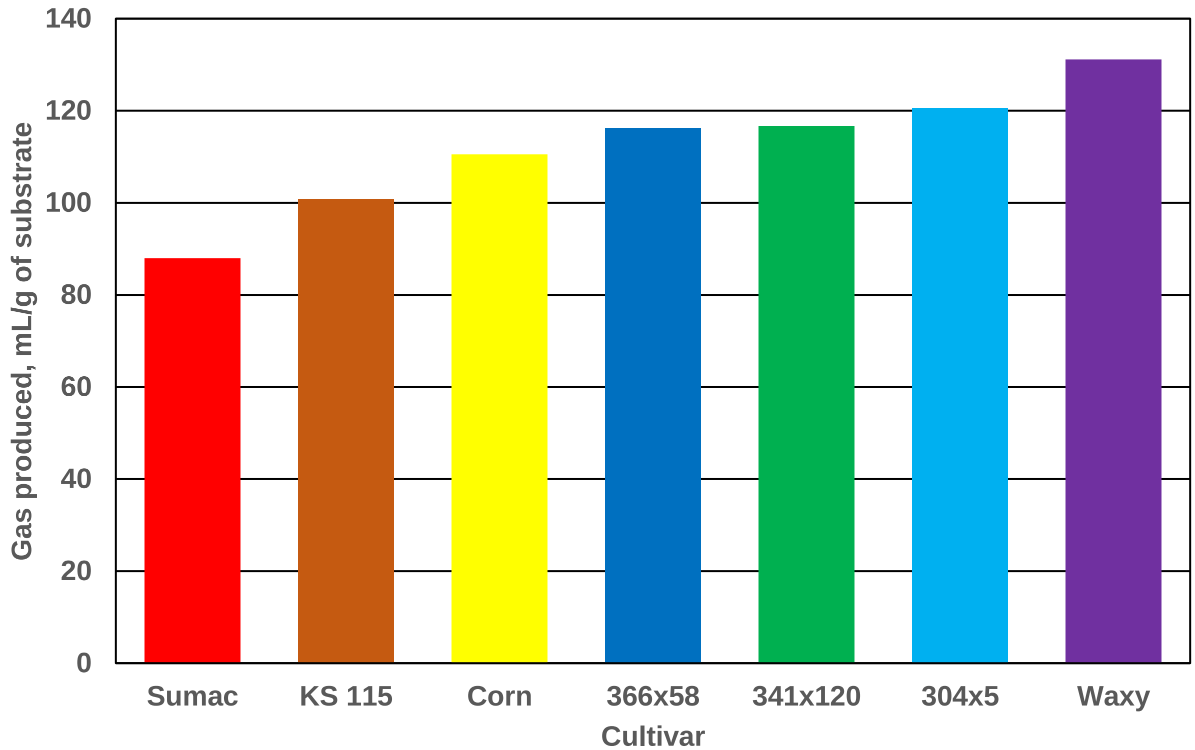
Figure 5. Gas production (experiment 2) by *in vitro* cultures using ground grains as substrate and ruminal fluid as microbial inoculum. SEM = 1.2. Effect of cultivar, P < 0.0001.



The *in vitro* screening data provided guidance in the selection of candidate cultivars for use in a cattle feeding study. Not all cultivars used in the screening work were available in quantities sufficient to perform a feeding trial, but two from the upper range of *in vitro* screening tests were identified-- a waxy sorghum and the 341x120 hybrid. The products were included in backgrounding diets consisting of 40% dry-processed grain, 30% alfalfa hay, 26% corn silage, and 4% supplement and fed to crossbreed beef steers (n=120) for 60 days using the Insentec feeding system that allowed for measurement of individual daily feed intakes. Results of the study are shown in Table 10. Feed intake was greater for cattle fed 341x120 sorghum compared to corn, and was intermediate for cattle fed wax sorghum, yielding greater average daily gain for both groups of cattle fed sorghum compared to those fed the corn-based diet. Feed efficiencies of sorghum-fed cattle were numerically improved, but differences were not significant. The study provides evidence that sorghum can be competitive with corn.

Table 10. Cattle performance during a 60-day backgrounding period when fed diets containing 40% dry-processed corn, 241x120 hybrid sorghum, or waxy sorghum.

	Grain Source				
Item	**Corn**	**341x120**	**Waxy**	***P*** **<**	**SEM**
No. animals	39	40	40	-	-
Initial BW, kg	272^{a}	271^{a}	276^{a}	0.2399	12.8
Final BW, kg	355^{a}	362^{ab}	369^{b}	0.0141	13.7
DMI, kg/d	8.79^{a}	9.48^{b}	9.30^{ab}	0.0397	0.245
ADG, kg	1.37^{a}	1.52^{b}	1.55^{b}	0.0055	0.041
Feed:gain	6.29	6.15	5.88	0.3585	0.220

^{a,b}Means within a row without a common superscript letter are different, $P < 0.05$.

Strategies for Control of Liver Abscesses

Cattle fed diets containing large proportions of highly fermentable carbohydrates are predisposed to development of liver abscesses. For more than four decades tylosin has been used as an effective strategy to reduce incidence of liver abscessed in feedlot cattle. There is increasing pressure to reduce antibiotic usage in livestock production systems, owing to concerns over development of antibiotic resistance. We have conducted a series of experiments aimed at reducing the use of in-feed antibiotics for liver abscess control. In the first of these experiments, crossbred steers (n=336) were fed diets with no tylosin, tylosin continuously throughout the 115-day trial, or tylosin fed only the final 34 days on feed. Feed intakes were greater for the two tylosin groups compared to the negative control (Table 11), but neither gain nor efficiency were different among treatments. Overall, abscess rate was relatively low, and was not different among groups. The continuous tylosin group had numerically fewer abscesses compared to the control, but this was not the case for the group fed tylosin only for the final 34 days on feed, suggesting exposure throughout the finishing phase may be necessary to reduce overall incidence.

Table 11. Finishing performance of steers (n=336) fed diets with no tylosin, continuous use of tylosin, or fed tylosin only during the final 34 days of feed.

Item	No tylosin	Tylosin fed Continuously[1]	Tylosin fed final 34 days[1]	SEM	*P* <
Initial BW, kg	456	460	458	6.4	0.79
Final BW, kg	627	631	625	7.9	0.76
DMI, kg/d	10.61[a]	10.94[b]	10.98[b]	0.116	0.01
ADG, kg	1.49	1.48	1.45	0.045	0.68
Feed:gain	7.14	7.41	7.58	0.233	0.19
Carcass weight, kg	392.7	394.4	388.8	4.13	0.23
Liver abscess, %	14.5	12.6	17.0	4.77	0.66
A-	9.2	6.2	9.8	3.72	0.51
A^0	1.8	3.6	2.7	2.20	0.72
A+	3.5	2.8	4.5	2.52	0.80

[1]Tylosin phosphate included in diets at 8 mg/kg dry matter.
[a,b]Means within a row without a common superscript letter are different, $P < 0.05$.

Our second experiment utilized 312 cross bred steers fed for 119 days. Steers were fed diets with no tylosin, tylosin fed continuously at the rate of 8 mg/kg diet dry matter, or tylosin fed in a predetermined intermittent use pattern. Cattle in the intermittent group received tylosin at 8 mg/kg diet dry matter throughout the 3-week step-up phase, and for the balance of the study tylosin was used in an off/on rotation in which it was removed from the diet for two weeks and included for one week. Overall, tylosin use for the intermittent group 60% less than that of the continuously-fed group. Feedlot performance, carcass weights, and liver abscess rates are shown in Table 12. Feed intake, average daily gain, efficiency, and carcass weights were similar among treatments. Cattle fed tylosin continuously or intermittently yielded similar improvements in reduction of liver abscesses compared to cattle in the control group.

Table 12. Finishing performance of steers (n=312) fed diets with no tylosin, continuous use of tylosin, or tylosin fed intermittently in a 1 week on two week off rotation for the duration of the finishing period.

Item	No tylosin	Tylosin fed Continuously[1]	Tylosin fed intermittently[2]	SEM	*P* <
Initial BW, kg	410	411	411	6.7	0.40
Final BW, kg	628	635	626	4.9	0.23
DMI, kg/d	10.89	11.23	10.86	0.235	0.28
ADG, kg	1.83	1.87	1.80	0.036	0.21
Feed:gain	5.96	6.01	6.04	0.097	0.75
Carcass weight, kg	380	383	380	6.1	0.51
Liver abscess, %	21.36[a]	7.84[b]	9.62[b]	4.655	0.01
A-	12.62	6.86	5.77	4.011	0.19
A^0	6.88	0.98	2.88	2.589	0.07
A+	1.94	0.00	0.96	1.378	0.37

[1]Tylosin phosphate included in diets at 8 mg/kg dry matter.
[2] For the intermittent treatment, tylosin was included in diets at 8 mg/kg dry matter during the step-up phase (initial three weeks on feed) and subsequently in rotations of 1 week on and 2 weeks off.
[a,b]Means within a row without a common superscript letter are different, $P < 0.05$.

Conclusions

- The commercialized strain of *Megasphaera elsdenii* can be used effectively to accelerate adaptation to highly fermentable diets. Benefits of the organism manifest in the form of extensive lactic acid metabolism, decreased incidence of acidosis, improved cattle health, increased gain, improvements in efficiency, improvements in carcass quality, and increased carcass value. Additionally, *Megasphaera* can be exploited as a tool to simplify feeding management, making it possible to decrease the number of diets used in the transition phase, decreased time to achieve top finishing rations, and decreasing manure output.
- High-amylase corn (Enogen Feed Corn) is promising as an energy source for cattle, improving feed efficiency while also reducing costs associated with grain processing.
- Sorghum grain cultivars are highly variable with respect to susceptibility to digestion by ruminal microorganisms. Cultivars are available that are competitive with corn and well-suited to use in cattle feeding.
- Tylosin is effective in controlling liver abscess incidence when fed in a discontinuous manner that results in substantial decrease in antibiotic usage.

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