



Microbes at Work!

Effect of Bio-Vet Microbial Combination Including *Propionibacterium* species Under In Vitro Closed System Rumen Simulation Conditions

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INTRODUCTION

Prior research has shown in vivo response to ruminal VFA levels and acidity when using a single *Propionibacterium* strain in beef heifers.

Bio-Vet's microbial formulations contain this same *Propionibacterium* strain in combination with other live microbial cultures, yeast and digestive enzymes. The objective of this study was to quantify the effects of Bio-Vet's microbial combinations on volatile fatty acid (VFA) production and total acidity under laboratory simulated, non-buffered, closed system, rumen conditions.

MATERIALS and METHODS

Fresh rumen fluid was collected from lactating dairy cows via rumen fistula. Fluid was strained and diluted to provide a final volume of 33% de-ionized water. Dextrose was added to 1.5% of fluid by weight. The resulting solution was transferred into flasks at 39° C with low agitation and constant flow CO₂ in the gas space above the liquid phase in the flask. One of three treatments was added to duplicated flasks. Treatments included: 1) control (no product added), 2) low dose of microbial product added, 3) high dose of microbial product added. The low and high dose rates represented the upper and lower range of normal feeding rates for various Bio-Vet microbial products containing this *Propionibacterium* strain.

Samples were collected from each flask at 0, 6, 12, 18, 24 and 48 hours. Each sample was analyzed for pH and the volatile fatty acids acetate, butyrate, iso-butyrate, lactate, propionate, valerate and iso-valerate using HPLC. The mean of the duplicated samples was used to compare treatments at various times.

RESULTS

Table 1 shows pH levels at various times for each treatment. As time progressed, pH dropped in each sample. Both samples treated with microbial cultures maintained a higher pH than the control sample at each time interval. The high dose microbial sample maintained a higher pH than the low dose microbial sample at each time interval. Figure 1 shows pH curves for each sample.

Table 2 shows individual and total VFA levels at each time interval. As time progressed, volatile fatty acids increased and accumulated in the closed system.

Lactate level in the control sample was higher than both samples containing microbial cultures at each time interval. The high dose microbial culture sample had lower lactate levels than the low dose microbial culture sample. Figure 2 shows lactate levels.

Acetate levels were similar for all treatments. Figure 3 shows acetate levels.

Propionate levels were higher in both samples containing microbial culture. The high dose microbial culture sample had higher propionate levels than the low dose

microbial culture sample at each time interval. Figure 4 shows propionate levels.

Butyrate levels were higher for both samples containing microbial culture than those of the control sample.

The high dose microbial culture sample contained higher total non-lactate VFA levels than the control and low dose microbial culture sample.

DISCUSSION

Acidity

Microbial culture samples had higher pH levels than the control sample. pH appeared dose dependent for the samples containing microbial culture. Some industry experts define acute rumen acidosis as the event occurring when rumen pH falls below 5.0. In this non-buffered, closed experimental system, the control sample pH fell below 5.0 between 6 and 12 hours after introduction of a highly fermentable carbohydrate source, dextrose; pH dropped as low as 4.33, and final pH was 4.36 at 48 hours. In contrast, pH in the samples treated with low dose and high dose microbial culture decreased slower and never dropped as low as the control sample pH. The lowest pH for the low dose microbial culture sample was 4.43, and final pH was 4.71. The lowest pH for the high dose microbial culture sample was 4.64, and final pH was 4.87. The absolute pH levels in this experiment can not be compared to those seen in the physiologically buffered *in vivo* rumen. The data does however suggest that the microbial culture used in this study could help prevent acute rumen lactic acidosis due to ruminal lactate accumulation.

Volatile Fatty Acids

Lactate was produced in each sample as the dextrose was rapidly fermented. However, in the samples containing microbial culture, lactate was decreased in later measurements. This data indicates that some portion of the microbial culture utilized the lactate. This is consistent with previous research on the *Propionibacterium* strain showing that the organism actually converts

lactate to propionate. This activity is further supported by the current study results since propionate levels were also higher for samples containing the microbial culture.

Lactate is very inefficient when absorbed by the cow and utilized as an energy source. Absorption rate of lactate across the rumen wall is relatively slow, leading to build up of lactate and subsequent decreased rumen pH when readily fermentable carbohydrates are fed.

In this study, acetate levels were maintained. Steady acetate levels in the presence of increased propionate and butyrate levels should increase milk production while maintaining milk solids when using this microbial culture.

Research with some strains of *Propionibacterium* have shown decreases in acetate levels and dramatic shifts in acetate:propionate ratios. Decreased acetate levels would be undesirable in dairy cows as they tend to reduce milk fat yield. Research with *Propionibacterium* strain P-63 has not shown any decrease in acetate levels. In cannulated rumen trials, when strain P-63 was fed for 7 days, acetate was actually increased compared to levels measured in the rumen of control animals. The *Propionibacterium* strains contained within the current microbial culture include strain P-63, and would be expected to maintain or increase acetate.

Research on feeding of yeast and yeast culture shows that they aid in maintaining rumen pH and increasing acetate levels. The inclusion of yeast in the microbial culture used in this study may have aided in maintaining acetate levels.

Increases in propionate levels for samples containing microbial culture are consistent with previous results when feeding *Propionibacterium* strain P-63. Utilization of lactate by the *Propionibacterium* strain increases the level of the more desirable VFA propionate. Propionate is more efficiently absorbed from the rumen and converted to energy (blood glucose) by the cow. This maintains a higher rumen pH and makes for

more energetic efficiency. Propionate is an efficient blood glucose precursor that does not lead to the accumulation of ketone bodies such as occurs when large amounts of body fat are mobilized during negative energy balance. For this reason, benefits could be expected from feeding the microbial culture during periods in which dairy cows are predisposed to ketosis.

Butyrate levels were increased for samples treated with microbial culture. The response was not dose dependent in this study. Butyrate is a highly usable VFA for lactating cows. It is efficiently absorbed and converted to milk production.

Mean iso-valerate level for the control sample at time = 18 hours was dramatically different than all other values. Review of the individual paired sample values showed one sample with a value of 0.365% and the second sample with a value of 0.029%. Since the highest individual iso-valerate level of all other samples was 0.052%, it appears that data was recorded with an error in decimal point placement. The 0.365% value most likely should have read 0.0365%.

The overall effect of the microbial culture used in this study was to decrease lactate, increase propionate and butyrate, with no negative effect on acetate levels. In lactating dairy cows, this alteration of VFA levels would be desirable. Figures 5, 6 and 7 show acetate, lactate and propionate levels for the low dose microbial culture, high dose microbial culture and control samples respectively. The data confirms that the intermediate fermentation acid, lactate, is converted to the final fermentation acid, propionate, by the microbial culture.

The high dose microbial culture was proportionally higher in each microbial component with the exception of *Propionibacterium*. One log₁₀ increase (10⁹ vs. 10¹⁰ CFU / head) in *Propionibacterium* occurred between the low and high dose microbial cultures in this study. A third feeding rate including one log₁₀ increase in *Propionibacterium* over the high dose used in this study

(10¹¹ CFU / head) showed even more dramatic improvements in pH and VFA levels. However, this feeding rate was deemed too expensive to feed routinely, and the data were not included in this report.

The low and high dose microbial cultures used in this study supplied 10⁵ to 10⁶ CFU per ml of rumen fluid. These levels have been shown effective in previous trials where *Propionibacterium* strains were fed.

The system used for this study most closely represents conditions of acute rumen acidosis following engorgement of rapidly fermentable carbohydrates such as might occur in grain overload conditions of ruminant nutrition (e.g. post calving dairy cows, incoming feedlot cattle, top dress grain fed cattle, etc.)

CONCLUSIONS

Microbial cultures used in this study, maintained a higher pH and improved VFA levels versus the control in the absence of physiological buffering that would normally occur in the natural rumen environment. Samples treated with a low dose of the microbial culture showed a 93% reduction in lactate levels versus the control sample. Samples treated with a high dose of microbial culture utilized 100% of the lactate under the simulated, closed system, rumen conditions of the study.

REFERENCES

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Table 1. Sample pH at various time intervals

Time	Control	Low	High
0	6.73	6.73	6.73
6	5.04	5.11	5.17
12	4.48	4.51	4.67
18	4.35	4.45	4.65
24	4.33	4.43	4.64
48	4.36	4.71	4.87

Table 2. Volatile fatty acid levels at various time intervals (percent)

Sample	Time	Lactate	Acetate	Propionate	I-Butyrate	Butyrate	I-Valerate*	Valerate	Total VFA	Total Non-Lactate VFA
Control	0	0.018	0.222	0.099	0.000	0.067	0.000	0.000	0.406	0.388
	6	0.094	0.279	0.177	0.000	0.112	0.000	0.000	0.660	0.566
	12	0.373	0.336	0.199	0.000	0.121	0.046	0.000	1.074	0.701
	18	0.379	0.327	0.219	0.000	0.101	0.197	0.000	1.223	0.844
	24	0.415	0.372	0.230	0.000	0.101	0.042	0.000	1.158	0.743
	48	0.340	0.356	0.261	0.000	0.118	0.035	0.000	1.108	0.768
Low	0	0.018	0.222	0.099	0.000	0.067	0.000	0.000	0.406	0.388
	6	0.096	0.286	0.199	0.000	0.098	0.000	0.000	0.678	0.582
	12	0.373	0.326	0.209	0.000	0.102	0.048	0.000	1.056	0.683
	18	0.341	0.313	0.231	0.000	0.099	0.035	0.000	1.018	0.677
	24	0.356	0.352	0.245	0.000	0.096	0.036	0.000	1.083	0.727
	48	0.024	0.372	0.333	0.000	0.196	0.044	0.000	0.968	0.944
High	0	0.018	0.222	0.099	0.000	0.067	0.000	0.000	0.406	0.388
	6	0.097	0.303	0.218	0.000	0.102	0.025	0.000	0.744	0.647
	12	0.323	0.337	0.233	0.000	0.117	0.045	0.000	1.053	0.730
	18	0.245	0.327	0.274	0.000	0.105	0.035	0.000	0.985	0.740
	24	0.243	0.353	0.290	0.009	0.109	0.040	0.000	1.043	0.800
	48	0.000	0.327	0.339	0.020	0.175	0.037	0.000	0.897	0.897

* T=18 hrs, possible reporting error for control sample

Figure 1. Sample pH versus time

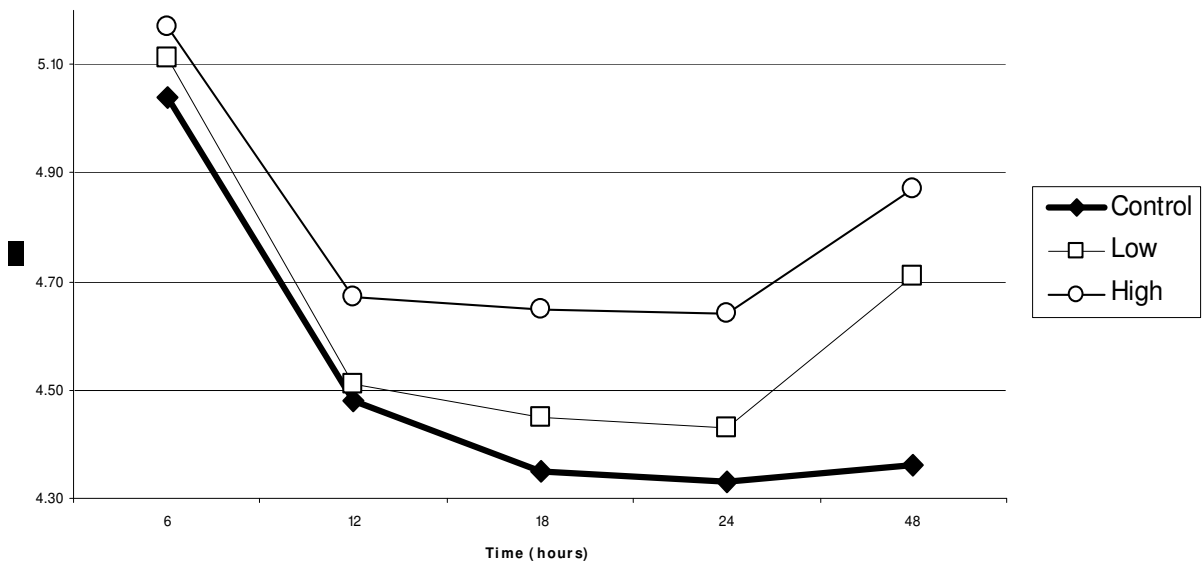


Figure 2. Lactate levels versus time

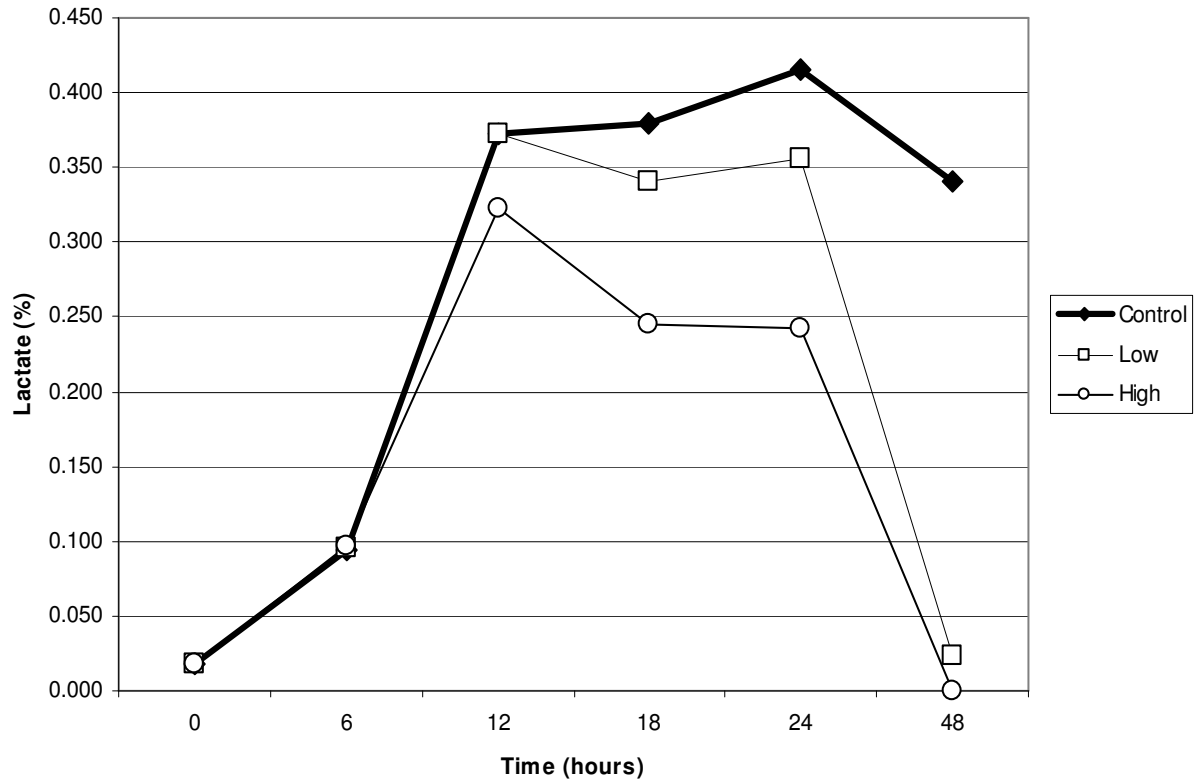


Figure 3. Acetate levels versus time

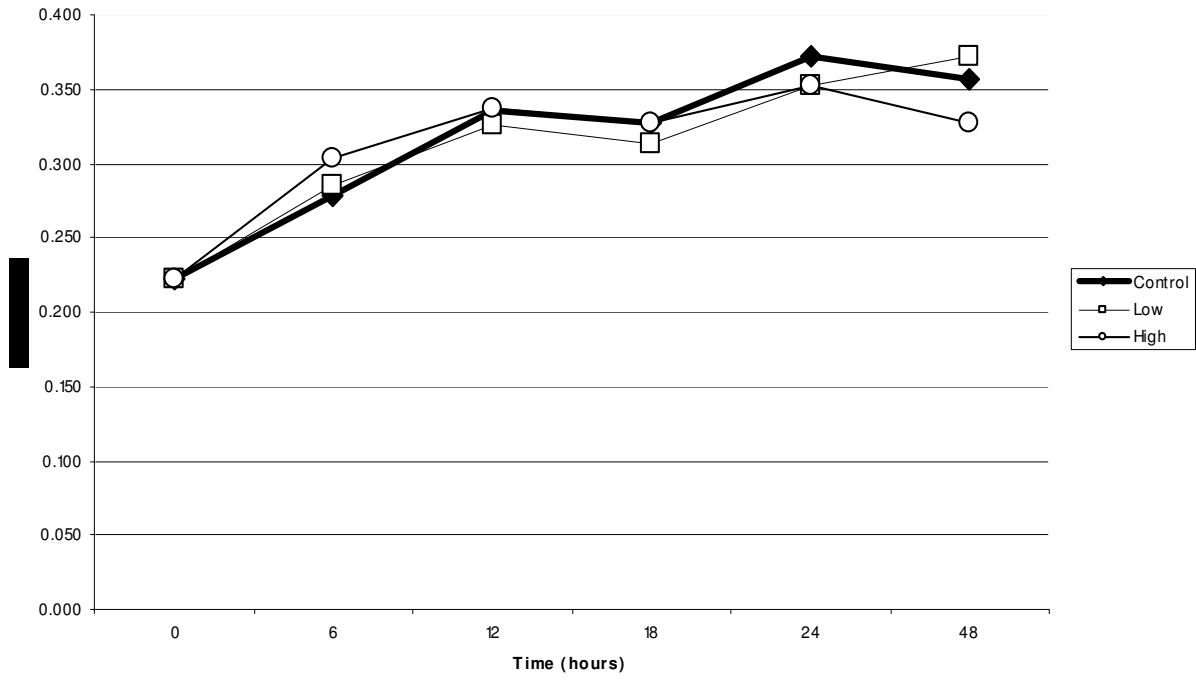


Figure 4. Propionate levels versus time

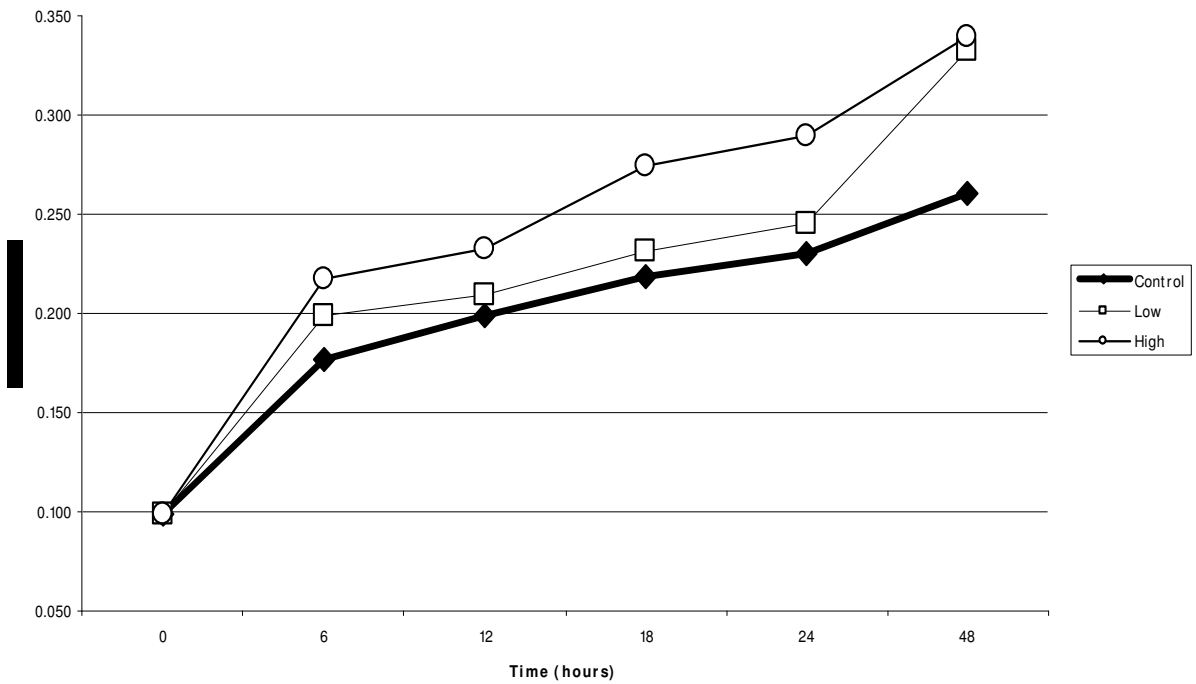


Figure 5. Low Dose Microbial Culture Sample acetate, lactate and propionate levels

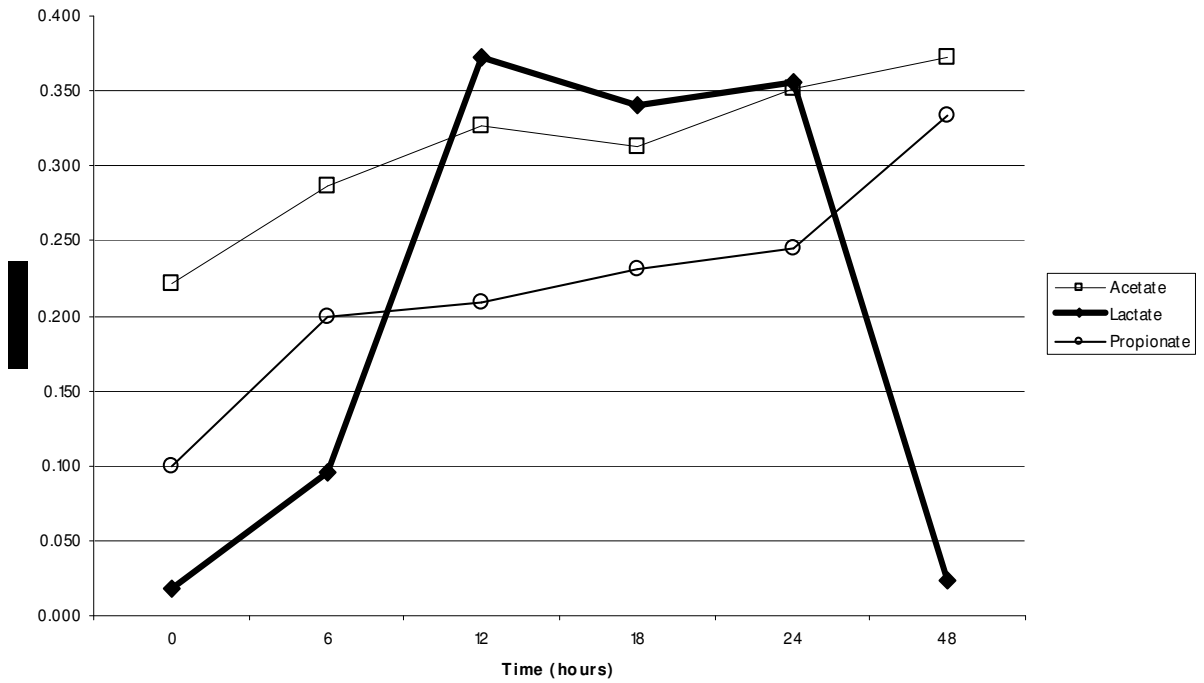


Figure 6. High Dose Microbial Culture Sample acetate, lactate and propionate levels

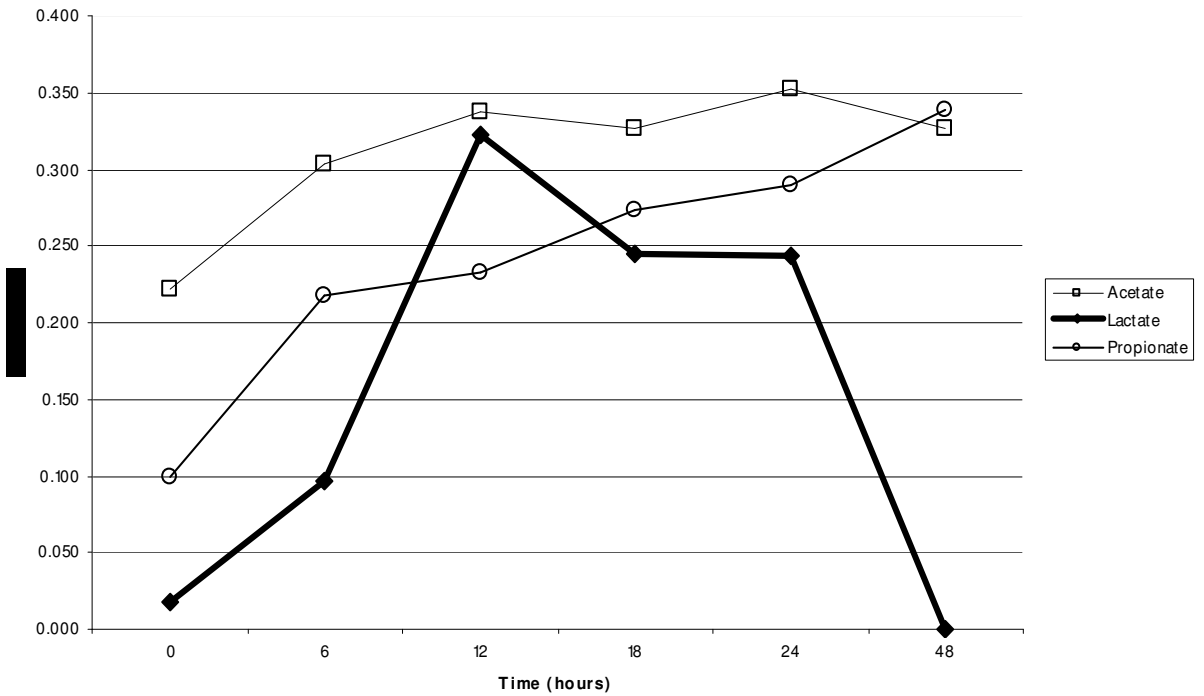


Figure 7. Control Sample acetate, lactate and propionate levels

