THE EVALUATION OF LIVE YEAST CULTURE ON THE PRODUCTION OF EARLY LACTATION DAIRY COWS

by

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ABSTRACT

The effects of the inclusion of a live yeast culture (LYC) containing *Saccharomyces cerevisiae* to ruminant diets was examined in two experiments. The first experiment was an *in vitro* study designed to determine the optimal level of inclusion of LYC to barley grain and orchardgrass hay as assessed by production of gas from fermentation. Parameters of cumulative, potential, rate and lag of gas production were measured. Gas production was monitored by computer interfaced pressure sensors every ten minutes for an incubation period of 24 h. Levels of LYC investigated were 0.0, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 % LYC on a dry matter (DM) basis of substrate. Rumen fluid inoculum was collected from two donor cows maintained on a 60:40 barley grain / orchardgrass hay diet. The barley grain portion of the ration was supplemented with 0.2 % LYC on a DM basis. The diet of the donor cows was then supplemented with 0.12 % LYC on a dry matter basis. Barley grain and orchardgrass hay samples were dried and ground through a 1 mm screen. Samples of either barley grain or orchardgrass hay, 150 mg, were incubated in quadruplicate, with 15 ml of rumen fluid and buffer solution in modified 50 ml Erlenmyer flasks. Gas production values were fitted to the following exponential growth model with lag \( Y = b \left( 1 - e^{-\alpha(t-h)} \right) \). Resulting gas production parameters indicated that for barley grain the 0.2 or 0.25 % LYC level would be the most suitable. These levels of LYC produced the highest potential gas production (15.80 and 16.89 ml/mg DM), the slowest rate of gas production (0.13 and 0.11 ml/h) and the shortest lag of fermentation (0.06 and 0.08 h), respectively. For barley grain the 0.10 % LYC inclusion level produced significantly (P<0.05) higher (389.2 mol) total VFA production when compared to the control (264.6 mol). The acetate, propionate levels and the acetate:propionate ratio were not affected (P>0.05) by the LYC inclusion. For orchardgrass hay, the 0.2 % LYC level produced a significantly (P<0.05) higher level of potential gas production (16.0 ml/mg DM) and decreased lag of onset of fermentation (0.20 h). The inclusion of LYC had no affect (P>0.05) on total VFA production.
Supplementation of LYC decreased acetate production when compared to the control for all levels of LYC. Inclusion of LYC had no effect (P>0.05) on propionate production and the acetate:propionate ratio. Based on the gas production experiment it was concluded that the 0.2 % level of LYC inclusion is optimal for a diet consisting of barley grain or orchardgrass hay.

The second experiment was an in vivo lactation study designed to determine the effect of LYC supplementation on dry matter intake (DMI) and milk yield of dairy cows in early lactation. Twenty-four multiparous cows were paired, based on age, body weight, previous year's 305 d milk production and then randomly assigned to one of two treatment groups, control or a diet supplemented with LYC. Pre partum DMI was monitored for approximately 7 days prior to parturition. Lactation was divided into two distinct periods; period one and period two. Period one, 0 – 14 days in milk (DIM), in which body condition score (BCS), body weight (BW), rumen pH, rumen volatile fatty acids, blood parameters, DMI, milk yield and milk composition were monitored. Period two lasted from 15 – 85 DIM in which milk yield and milk composition were recorded. Pre partum cows were fed a basal ration consisting of 85 % forage and 15 % concentrate on a DM basis. In addition to the basal ration all cows received 1 kg of concentrate, twice daily, fed separately from the basal ration. LYC was fed at a level of 0.2 % of the total diet on a DM basis and fed in two equal portions that were hand mixed into the separately fed concentrate. Post partum cows received the basal ration with separately fed incremental increases in concentrate of 0.5 kg/d until 14 DIM. Results indicated that LYC had no significant (P>0.05) effect on pre partum or period one DMI, milk composition, ruminal pH, selected blood parameters, body weight (BW) or body condition score (BCS). During the first fourteen days of lactation there were no significant (P<0.05) difference in milk yield between the control and the supplemented group except for day 14 (42.2 vs. 37.5 kg/d). Analysis of volatile fatty acids for period one indicated that the inclusion of LYC had no significant (P>0.05) effect on total or individual volatile fatty acid production. During period two, in which there was no LYC
supplementation cows, that previously received LYC showed a significant (P<0.05) increase in milk yield and higher peak lactation when compared to the control group (44.1 vs. 40.4 kg/d). These studies demonstrate that the inclusion of LYC prior to parturition is beneficial for cows in early lactation.
TABLE OF CONTENTS

TITLE ................................................................. I

ABSTRACT ............................................................ II

TABLE OF CONTENTS ................................................ V

LIST OF TABLES .................................................... VIII

LIST OF FIGURES ................................................... X

LIST OF ABBREVIATIONS ........................................... XI

ACKNOWLEDGEMENTS .............................................. XII

1.0 GENERAL INTRODUCTION ..................................... 1

1.1 YEAST ............................................................ 1

1.1.1 RESPONSES TO YEAST SUPPLEMENTATION ............. 2

1.1.2 MODE OF ACTION OF LYC ............................... 5

1.1.3 CYC CONCEPT .............................................. 6

1.1.4 OBJECTIVES OF THE THESIS ............................ 7

1.3 REFERENCES ................................................... 8

2.0 EFFECT OF LIVE YEAST CULTURE ON THE IN VITRO GAS PRODUCTION OF BARLEY GRAIN AND ORCHARDGRASS HAY .................................................. 13

2.1 ABSTRACT ....................................................... 13

2.2 INTRODUCTION .................................................. 16

2.2.1 IN VITRO TECHNIQUES .................................... 17

2.2.2 VALIDATION OF THE GAS MEASUREMENT MACHINE .... 18

2.3 MATERIALS AND METHODS .................................. 19

2.3.1 VIABILITY OF YEAST CULTURE .......................... 19

2.3.3 TREATMENTS .............................................. 20

2.3.5 ANIMALS AND FEEDING ................................ 20

2.3.6 RUMEN FLUID COLLECTION .............................. 21

2.3.7 GAS MEASUREMENT MODIFICATIONS .................. 21

2.3.8 PROCEDURES FOR GAS MEASUREMENT ............... 22

2.3.9 COLLECTION OF GAS DATA ............................. 22

2.3.10 CALIBRATION OF GAS MEASUREMENT MACHINE .... 23

2.3.11 ANALYSIS OF GAS DATA ............................... 24

2.3.12 VOLATILE Fatty ACID ANALYSIS .................... 25

2.3.13 STATISTICAL ANALYSIS ................................ 25
2.4 RESULTS

2.4.1 CUMMULATIVE GAS PRODUCTION

2.4.2 POTENTIAL GAS PRODUCTION

2.4.3 RATE OF GAS PRODUCTION

2.4.4 LAG OF FERMENTATION

2.4.5 VOLATILE FATTY ACIDS

2.6 DISCUSSION

2.7 CONCLUSION

2.8 REFERENCES

3.0 EFFECT OF LIVE YEAST CULTURE SUPPLEMENTATION ON DRY MATTER INTAKE AND MILK PRODUCTION OF EARLY PRODUCTION COWS

3.1 ABSTRACT

3.2 INTRODUCTION

3.3 MATERIALS AND METHODS

3.3.1 EXPERIMENTAL DESIGN

3.3.2 YEAST ALLOTMENT

3.3.3 DIET

3.3.4 MEDICAL TREATMENTS

3.3.5 FEED SAMPLING

3.3.6 BLOOD SAMPLING AND ANALYSIS

3.3.7 RUMEN FLUID SAMPLING

3.3.8 MILK SAMPLING

3.3.9 EFFICIENCY

3.3.10 BODY CONDITION SCORE

3.3.11 STATISTICAL ANALYSIS

3.4 RESULTS

3.4.1 FEED COMPOSITION

3.4.2 DIETARY INTAKES

3.4.3 BLOOD COMPOSITION

3.4.4 RUMEN FLUID pH

3.4.5 VOLATILE FATTY ACIDS

3.4.6 MILK PRODUCTION PERIOD ONE (0 – 14 DIM)

3.4.7 MILK PRODUCTION PERIOD TWO

3.5 DISCUSSION

3.6 ECONOMIC BENEFITS

3.7 CONCLUSIONS

3.7 REFERENCES
4.1 FUTURE RESEARCH .................................................................................. 88

4.2 GENERAL CONCLUSIONS ...................................................................... 88

APPENDIX 1.0 COMPOSITION OF REDUCING SOLUTION FOR IN VITRO GAS PRODUCTION ................................................................. 90

   MAIN ELEMENT SOLUTION .................................................................. 90
   TRACE ELEMENT SOLUTION ................................................................. 90
   BUFFER SOLUTION .............................................................................. 90
   REDUCING SOLUTION ....................................................................... 91
   REFERENCE .......................................................................................... 91

APPENDIX 2.0 REGRESSION ANALYSIS ..................................................... 92

APPENDIX 3.0 ECONOMIC BENEFITS FOR PRODUCTION TRIAL .......... 93
LIST OF TABLES

TABLE 2.1  Effect of LYC supplementation on gas measurement parameters of barley grain. .......................................................... 38

TABLE 2.2  Effect of LYC supplementation on gas measurement parameters of orchardgrass hay. .......................................................... 39

TABLE 2.3  Effect of all levels of LYC on 0 H and 24 H volatile fatty acid production from barley grain. .......................................................... 40

TABLE 2.4  Effect of all levels of LYC on 0 H and 24 H volatile fatty acid production from orchardgrass hay. .......................................................... 41

TABLE 2.5  Effect of LYC on volatile fatty acid production for all levels of LYC from barley grain. .......................................................... 42

TABLE 2.6  Effect of LYC on volatile fatty acid production for all levels of LYC from orchardgrass hay. .......................................................... 43

TABLE 3.1  Nutrient composition (DM basis) of feeds from February 14 until April 18, 1997 .......................................................... 71

TABLE 3.2  Nutrient composition (DM basis) of feeds from April 18 until July 17, 1997 .......................................................... 72

TABLE 3.3  Effect of LYC on pre partum dry matter intake. ................................. 73

TABLE 3.4  Calculated amounts of LYC for LYC supplemented cows. ................................. 74

TABLE 3.5  Influence of yeast supplementation on dry matter intake, body condition score and body weight for period one. ................................................. 75

TABLE 3.6  Effect of LYC supplementation on selected blood parameters for period one. .......................................................... 76

TABLE 3.7  Effect of LYC supplementation on rumen fluid pH and average volatile fatty acid concentration for period one. .......................................................... 77

TABLE 3.8  Effect of LYC supplementation on volatile fatty acid concentration for week one of period one. .......................................................... 78

TABLE 3.9  Effect of LYC supplementation on volatile fatty acid concentration for week two of period one. .......................................................... 79

TABLE 3.10  Milk yield and composition for period one. .......................................................... 80

TABLE 3.11  Effect of LYC supplementation on individual 14 d milk yield in period one. .......................................................... 81
TABLE 3.12  MILK YIELD AND COMPOSITION FOR PERIOD TWO. 82

TABLE 3.13  AVERAGE WEEKLY MILK YIELD FOR PERIOD ONE AND PERIOD TWO. 83
LIST OF FIGURES

FIGURE 3.1 EFFECT OF YEAST SUPPLEMENTATION ON DAILY DRY MATTER INTAKE FOR PERIOD ONE. 84

FIGURE 3.2 EFFECT OF LYC SUPPLEMENTATION ON DAILY MILK YIELD FOR PERIOD ONE 85

FIGURE 3.3 EFFECT OF LYC SUPPLEMENTATION ON AVERAGE MILK WEIGHT FOR PERIOD ONE AND PERIOD TWO 86

FIGURE 3.4 EFFECT OF LIVE YEAST CULTURE ON 305 D MILK YIELD 87
LIST OF ABBREVIATIONS

d  day
h  hour
BCS  body condition score
BW  body weight
CYC  Choong Yeast Culture
DIM  days in milk
DM  dry matter
DMI  dry matter intake
LSM  Least square means
LYC  live yeast culture
SC  *Saccharomyces cerevisiae*
SE  Standard error
VFA  volatile fatty acid
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1.0 GENERAL INTRODUCTION

1.1 YEAST

For years ruminant nutritionists have attempted to manipulate the microbial ecosystem of the ruminant with hopes of increasing production. Currently, the most common means for enhancing ruminant production is through the use of chemical feed additives. The mode of action of these additives is through the manipulation of ruminal fermentation patterns. These additives, such as antibiotics and ionophores, have antimicrobial activity which enables them to eliminate specific organisms in the rumen. On the part of both the consumer and the producer, there is a growing concern with the use of antibiotics and growth stimulants in the animal feed industry due to the possibility of chemicals entering into the food chain (Chiquette 1995).

With the increasing concern about residual effects of hormone and antibiotic additives, alternative enhancers have been sought. In the past 20 years, research has shown beneficial results with the inclusion of live yeast cultures in lactating dairy cow rations. The low manufacturing costs associated with the production of yeast cultures have contributed to their use in the animal production industry. In contrast to chemical feed additives, yeast cultures stimulate the growth of specific rumen bacteria (Wallace 1993), which has resulted in a renewed interest in the use of yeast as a natural, safe and cost effective feed additive.

Yeast, commonly *Saccharomyces cerevisiae* (SC), has been utilized successfully for years in both human and animal industries. The most common uses of yeast in the human industry have been in brewing, distilling and baking. These processes produce tonnes of waste yearly and the removal of this waste is not only time consuming but costly. Analysis of these waste by-products have shown that they are rich in nutrients and vitamins which can be utilized as supplements for both ruminants and monogastrics. Yeast by-products were first utilized in dairy rations as alternative protein sources (Girard and Dawson 1995). In 1925, Williams first reported the use of yeast in the diet of lactating dairy cows (Williams and Newbold 1990). The
supplementation of LYC is commonly fed in the rations of early to mid lactation dairy cows, starting from seven to twenty one days into lactation. The inclusion of LYC could be more beneficial if feed prior to parturition in the transition period. The transition period lasts from seven days prior to parturition and extends to fourteen days into lactation. These transition dairy cows could benefit from the inclusion of LYC in their ration.

Active live yeast culture first received attention as a feed additive in the dairy industry in the 1940's and 1950's. Renz in 1954 included 50g/d/head of live yeast culture (LYC) in ruminant rations which resulted in an increase of milk production by 1.1 kg/day (Williams and Newbold 1990, Moloney and Drennan1994). Inclusion of LYC in dairy rations elicits a variety of responses ranging from increased milk yield to changes in the volatile fatty acid (VFA) profile. These production responses depend on a variety of parameters such as the activity of the yeast, diet composition, feeding system and type of yeast strain (Stewart 1995).

1.1.1 RESPONSES TO YEAST SUPPLEMENTATION

Previous studies (Hoyos et al., 1987, Weidmeier et al., 1987, Dawson 1988, Harrison et al., 1988, and Williams 1989) have shown a variety of beneficial results from the inclusion of LYC in lactating dairy cow rations. The most consistent result from the LYC inclusion was an increase in milk production as seen by Hoyos et al., 1987, Gunther 1989, Williams 1989, Williams and Newbold 1990, Williams et al., 1991 and Erasmus et al., 1992. Other studies have shown no change in milk yield (Erdman and Sharma 1989, Swartz et al., 1994, Chiquette 1995 and Besong et al., 1996). Other benefits seen from LYC supplementation were an increase in ruminal bacteria numbers (Weidmeier et al., 1987, Harrison et al., 1988, Dawson 1990, Offer 1990, Williams and Newbold 1990, Edwards 1991, Nisbet and Martin., 1991, Williams et al., 1991, Newbold et al., 1992 and Dawson 1993), stabilization of the ruminal pH.
(Dawson and Newman 1987), decrease in ruminal lactic acid concentration (Harrison et al., 1988, Martin et al., 1989 and Williams and Newbold 1990), rise in ruminal acetate concentrations (Harrison et al., 1988 and Callaway and Martin 1997), change in acetate:propionate (A:P) ratio (Harrison et al., 1988), increased fiber digestion (Newbold et al., 1991, Callaway and Martin 1997, Robinson 1997), increased feed efficiency (Kamalamma et al., 1996), and an increase in dry matter intake (DMI) (Ruf et al., 1953, Phillips and Vontungelin 1985, Harris and Lobo 1988, Williams et al., 1991, Wohlt et al., 1991). Other studies have shown that the inclusion of LYC in the dairy ration had no effect on ruminal parameters or milk production (Weidmeier et al., 1987, Adams et al., 1981, Erdman and Sharma 1989, Arambel and Kent 1990, Chademana and Offer 1990 and Swartz et al., 1994.). There have been no studies to date that have indicated a negative response to the addition of LYC to dairy rations. Inconsistencies in the results of previous studies may be explained by differences in methodology. These differences include the stage of lactation (Grings et al., 1992, Kung et al., 1997), ratio of concentrate to forage (Erfle et al., 1982, Kamalamma et al., 1996), feeding system (Erfle et al., 1982, Kamalamma et al., 1996), type of yeast strain (Newbold et al., 1995), growth medium of LYC (Newbold et al., 1995), how the yeast was fed (Kung et al., 1997), amount of yeast applied to the diet (Yoon and Stern 1996) and the activity of the yeast culture (Newbold et al., 1995).

Benefits of LYC supplementation in dairy rations can be associated with the stimulatory effect on specific groups of microorganisms in the rumen. LYC can selectively stimulate two types of bacteria; lactic acid utilising and cellulose digesting. This ability of LYC is not common to all strains of yeast but it does help explain some of the variations seen in animal response (Williams 1989).

The transition from late pregnancy to early lactation is a nutritionally demanding time for the dairy cow (Bell 1995). Pre partum cows are under considerable nutritional stress due to
rapid fetal growth and mammary development (Grummer 1995). Robinson (1997) and Grummer (1995) observed a decrease in DMI by 30 – 40 % a few days prior to calving. This decrease in DMI, at a time when nutrient demands are increasing, can lead to a negative energy balance forcing cows to rely heavily on their body reserves. This decreased feed intake can have a serious effect on reproductive and lactational performance (Erb et al., 1985, Pedron et al., 1993). The cause of such a dramatic decrease in DMI is not known, although there is speculation that it is driven by the hormone estrogen (Vazquez-Anon et al., 1994) or by physiological mechanisms such as rumen fill (Johnson and Combs 1992, Grant and Albright 1995). Monitoring and maintaining DMI during this stressful period would make the transition from pregnancy to lactation easier by minimizing nutritional stress, maximizing energy intake and lowering fatty acid mobilization (Grummer 1995).

It is possible that the results seen from the inclusion of LYC in lactating dairy cow rations could be beneficial to the transition dairy cow. The transition period can be defined as the time frame from 7 d pre partum until two weeks post partum. Inclusion of LYC is most common in diets of early to mid lactation cows (Grings et al., 1992, Piva et al., 1993, Chiquette 1995 and Kung et al., 1997) and fed for increased milk yield, stabilized ruminal pH, increased bacterial count and a change in VFA production. It is plausible that these results could be beneficial to the dairy cow in the transition period, but there has been little work done with the transition cows rations. Previous work has included LYC in the ration from 21 d pre partum until 14 d post partum (Robinson 1997) and from 30 d prior to parturition through 18 weeks of lactation (Wohlt et al., 1991). These studies have shown that responses to LYC supplementation are inconsistent. Robinson (1997) showed that LYC had no affect on pre or post partum DMI, while Wohlt et al, (1991) showed no change in pre partum DMI but an increase in post partum DMI. The lack of consistent results and the potential for benefits arising from feeding LYC in the pre partum period represents an area for further research.
1.1.2 MODE OF ACTION OF LYC

To date the exact mode of action of yeast cells in the rumen is not known. However, previous studies, both in vitro and in vivo, have produced many existing proposed actions of yeast in the rumen (Williams and Newbold 1990, Dawson 1990, Offer 1990, Erasmus et al., 1992 and Girard and Dawson 1995). *Saccharomyces cerevisiae* can survive in an anaerobic environment, such as the rumen, because it is a facultative anaerobe. The ability of the yeast to endure the harsh conditions of the rumen is an essential attribute to its effectiveness. Presence of viable yeast cells in the rumen fluid and feces are an indication that the supplement of LYC cells are capable of surviving the rumen (Kamalamana, et al., 1996). Yeast cells must remain metabolically active, but not reproductively active in order to survive and elicit a response in the rumen of dairy cows (Dawson 1993 and Stewart 1995). To ensure that active yeast cells reach the rumen it is necessary that yeast supplements contain viable yeast cells. Newbold et al., (1995) found that when non-viable yeast cells were included in the ration of dairy cows no responses were noted.

A common effect of feeding yeast to ruminants is the stabilization of the rumen environment. The presence of LYC in the rumen stimulates growth of specific bacteria such as lactate and cellulolytic utilizing bacteria (Callaway and Martin 1997). Lactate utilizing bacteria convert lactic acid to propionic acid, which is then utilized by the cow as energy. The decrease in lactic acid concentration in the rumen raises the pH and creates a more stable rumen environment (Dawson 1990).

Another effect is an increase in the total numbers of microbes in the rumen (Weidmeier et al., 1987, Harrison et al., 1988, Dawson 1990, Newbold et al., 1992 and Dawson 1993). Increases in cellulolytic bacteria could cause an increase in the rate of cellulose degradation, which would then stimulate DMI intake of dairy cows and result in a greater nutrient supply for
the production of milk (Stewart 1995). It is also believed that yeast cultures provide soluble
growth factors such as B vitamins, proteins and other nutrients. Nutrients such as these can be
utilized by the rumen microbes for growth and maintenance (Callaway and Martin 1997). Yeast
cells can autolysis and release growth factors that the bacteria of the rumen can utilise for
maintenance (Piva et al., 1989).

Other beliefs are that LYC can act as an oxygen scavenger creating a more anaerobic
environment, which is favourable for rumen micro-organisms. Yeasts are microaerobes and
consume oxygen (Rose 1987). A decrease in free oxygen concentration in the rumen can
improve the anaerobic environment (Rose 1987). All of these theories enhance the rumen
microecosystem, improve rumen digestion and contribute to the beneficial results seen in
lactating dairy cows.

1.1.3 CYC CONCEPT

The Korean based company Choong Ang Chemical Company Ltd. has developed a
Saccharomyces cerevisiae live yeast culture, (SC LYC), which they believe to be beneficial for
both ruminant and monogastric diets. Although responses to the inclusion of SC LYC have not
been scientifically documented, they have been reported as developer advertising claims.
Choong Ang Chemical Company Ltd. claims to have developed a method for producing live
yeast cultures which is different from those in North America. The yeast is dried in such a
manner to preserve the fermenting activity, while avoiding damage to the cells. Choong Ang
Chemical Company Ltd. yeast product is an active live yeast culture plus the media in which it
is grown. This live yeast product is called Choong Yeast Culture (CYC). As reported in the
developer advertising claims, CYC has been fed successfully in swine, poultry and dairy
industries in South Korea and the results indicate a reduction in production costs with higher
economic returns. The postulated mode of action of CYC is through improved nutrient
availability to the animal. Choong Ang Chemical Company Ltd. claims that CYC can stabilize intestinal bacteria, enhance numbers of anaerobic bacteria, alter fermentation patterns and change VFA ratios. Overall Choong Ang Chemical Company Ltd. claims that CYC will benefit the production of monogastrics and ruminants.

1.1.4 OBJECTIVES OF THE THESIS

1) To determine the optimal inclusion level of live yeast culture to barley grain and orchardgrass hay as assessed by \textit{in vitro} gas production.

2) To determine the effect of live yeast culture supplementation on dry matter intake and milk production of early lactation dairy cows.
1.3 REFERENCES


2.0 EFFECT OF LIVE YEAST CULTURE ON THE *IN VITRO* GAS PRODUCTION OF BARLEY GRAIN AND ORCHARDGRASS HAY

2.1 ABSTRACT

Incorporation of live yeast culture (LYC) in ruminant diets has been reported to increase milk production. The level of LYC inclusion used in dairy rations has been inconsistent. The objective of this study was to determine the optimal inclusion level of LYC to barley grain and orchardgrass hay assessed by *in vitro* gas production. Rumen fluid was collected from two cows maintained on a 60:40 barley grain/orchardgrass hay diet. The barley grain portion of the ration was supplemented with 0.12 % LYC on a dry matter (DM) basis. The LYC was added to the concentrate portion of the diet at a level of 0.2% LYC on a dry matter basis. Approximately 150 mg of each substrate, either barley grain or orchardgrass hay, dried and ground through a 1 mm screen, was incubated in quadruplicate with 15 ml of rumen fluid and buffer solution. Gas production was monitored with a computerized interfaced system using pressure sensors, every 10 min for an incubation period of 24 h. Levels of LYC investigated were 0.0, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30% of the DM of the substrate. Resulting gas production was fitted to the following exponential growth model with lag (\( Y = b \left( 1 - e^{-ct} \right) \)), where \( Y \) is the cumulative or 24 h total gas production produced at time \( t \), \( b \) is the potential gas production produced, \( c \) is the rate of gas production and \( l \) is the initial lag prior to the onset of gas production. No significant (\( P>0.05 \)) differences were noted in cumulative gas production from barley grain when the control was compared to LYC treatments. However, when compared to the control, all levels of LYC, except 0.10 %, had significantly (\( P<0.05 \)) greater potential gas production. Significantly (\( P<0.05 \)) higher amounts of potential gas (15.80 and 16.89 mg/ml) were produced from the 0.2 and 0.25 % LYC inclusion levels, respectively. The most significant (\( P<0.05 \))
decrease in rate of gas production (0.13 and 0.11 ml/h) was seen at the 0.2 and 0.25 % LYC levels when compared to the control (0.21 ml/h). All levels of LYC inclusion significantly (P<0.05) decreased the lag of fermentation when compared to the control. A significant (P<0.05) reduction in the lag of fermentation (0.06 and 0.08 h) was seen for the 0.2 and 0.25% LYC inclusion levels in comparison to the control (0.47 h). The cumulative gas production results for orchardgrass hay were similar to those observed for barley grain. No significant (P>0.05) differences were seen in the cumulative gas production when compared to the control. Potential gas production was significantly (P<0.05) higher for all levels of LYC inclusion when compared to the control. The highest potential gas productions (16.09 and 16.75 g/ml DM) were seen at the 0.2 and 0.3 % LYC inclusion levels, respectively. All levels of LYC inclusion significantly (P<0.05) reduced the rate of gas production from orchardgrass hay. The fastest rate of gas production (0.118 ml/h) for orchardgrass hay was seen at the 0.25 % LYC level. This was significantly (P<0.05) lower than the control (0.12 ml/h). All levels of LYC inclusion, except for the 0.30 % level, significantly (P<0.05) decreased the lag of fermentation when compared to the control. The lowest lag times (0.35, 0.35 and 0.20 h) were observed at the 0.10, 0.15 and 0.20 % LYC inclusion level in comparison to the control (0.95 h). For barley grain VFA analysis indicated that there was a significant (P<0.05) increase in total VFA production for the 0.10 % LYC inclusion level when compared to the control (389.3 vs. 264.6 mols respectively). Both the 0.2 and 0.25 % LYC inclusion levels resulted in total VFA productions similar to the control (258.6, 304.5 and 264.6 mols respectively). LYC had no significant (P>0.05) effect on acetate or propionate concentrations or on the acetate:propionate ratio. There was no significant (P>0.05) change in total VFA or propionate production for any level of LYC inclusion in orchardgrass hay. Propionate levels were numerically higher than the control for all levels of LYC inclusion. Molar percentage for both acetate and acetate:propionate ratios were numerically lower than the control. Acetate
percentages were significantly (P<0.05) lower for all levels of LYC, except the 0.3 % level, when compared to the control. This work indicates that the inclusion of 0.2 or 0.25 % level of LYC would stimulate potential gas production and decrease lag as well as the rate of fermentation of barley grain when incubated in vitro. In the case of orchardgrass hay, inclusion of either 0.2 or 0.30 % LYC appears to have the potential to improve the fermentation pattern of the in vitro system compared to the control.
2.2 INTRODUCTION

Many attempts have been made to modify the ruminal fermentation patterns of the lactating dairy cow to enhance milk production. Research has demonstrated live yeast culture (LYC) to be a natural and safe feed additive for use in dairy rations. Inclusion of LYC in rations has elicited a variety of responses from both \textit{in vitro} and \textit{in vivo} studies. Results seen from LYC inclusion can be contradictory due to the differences in yeast strains and methodology among the studies. Use of \textit{in vitro} experimentation can help to explain inconsistencies found in LYC supplementation studies.

\textit{In vitro} techniques can be used as a means of collecting information and then applying this knowledge to the \textit{in vivo} system. \textit{In vitro} procedures are controllable systems which can assist researchers in learning about and explaining the dynamic animal. Differences between laboratory simulation and animal experimentation can be reduced but may never be eliminated. With advances in technology, \textit{in vitro} techniques are becoming more reliable and therefore more comparable to \textit{in vivo} results. Menke et al., (1979) developed an \textit{in vitro} measurement system which measures the amount of gas released from feedstuffs incubated with rumen fluid. The authors also demonstrated that there was a high correlation between the amount of gas released and the digestibility of feedstuffs. Their \textit{in vitro} methods were found to be more accurate than other methods such as the two stage \textit{in vitro} method of Tilley and Terry (1963) or the cellulase method of Kellner and Kirchgessner (1976) (Menke et al., 1979, Menke and Steingass 1988, and Theodorou et al., 1994). This high correlation was seen because the gas measurement system is not based on a filtration process, which is needed for the separation of digested and undigested material in other \textit{in vitro} techniques (Menke et al., 1979). Over the last two decades, the gas measurement system has undergone modifications and simplifications which have made it a more useful tool in ruminant nutrition studies. Schofield et al., (1994) modified the original method of Menke et al., (1979) to include computer interfaced pressure
sensors. Still further modifications have been developed at the University of British Columbia such as simplified mixing and sampling techniques. There has been a renewed interest in in vitro gas measurement in the past few years because of these modifications that have made it more user friendly.

With the variety of beneficial responses recorded with LYC supplementation, it seems apparent that there might exist an optimal level of LYC inclusion. The inclusion level of LYC employed in the rations of previous studies ranged from 10-90 g/day/head (Arambel and Kent 1990 and Harris et al., 1992). No response was seen from the lower or upper level of LYC inclusion. The use of the gas measurement in vitro system allows for the examination of the effect of adding varying levels of LYC. The most effective level of LYC would then be applied to a subsequent in vivo experiment.

The objective of this study was to determine the optimal inclusion level of live yeast culture to barley grain and orchardgrass hay assessed by in vitro gas measurement.

2.2.1 In Vitro Techniques

In vitro techniques have gained interest in the last few years. Interest has been focused on development of techniques and procedures that will produce similar results to in vivo procedures. In vitro techniques have the ability to evaluate the effects of feed additives under strictly controlled conditions. These effects on rumen fermentation can be quantitatively monitored and evaluated (Gray and Ryan 1987). Researchers tend to move away from in vivo rumen incubation techniques because they are expensive and labour intensive trials to run, while in vitro rumen fluid incubation techniques are less expensive and less laborious to conduct. In vivo rumen incubation trials require large amounts of time, allocated to preparation of samples and care of donor animals. In vitro incubation trials are much quicker to perform, with less preparation and animal care required. A specific limitation of the in vivo technique is the small
number of samples that can be processed at one time. With *in vivo* work, specifically *in situ*, there must be a large supply of substrate because the amount required for each evaluation is large. These limitations are in contrast to the *in vitro* method in which numerous samples can be run with a very small quantity of substrate. With the use of an *in vitro* system, there is less stress on donor animals so from an animal welfare point of view the *in vitro* systems are better. However, a criticism of *in vitro* systems is the comparability of the results to those found *in vivo*. A question has been raised as to whether or not results from closed *in vitro* systems can be applied to the dynamic animal system. Overall the use of *in vitro* work can be very beneficial because standard techniques and apparatus can be developed and utilized in laboratories around the world. If the same procedures are followed, reliable, repeatable methods can be used for a variety of samples over a broad range of conditions.

### 2.2.2 Validation of the Gas Measurement Machine

*In vitro* gas measurement techniques are designed for the evaluation of feed digestion by ruminal microorganisms and are largely accepted because they are highly correlated with *in vivo* digestion (Schofield and Pell 1995 and Theodorou et al., 1994). Errors arise from misreading gas volumes and gas leakage problems (Schofield and Pell 1995). The potential for errors associated with manual methods of gas collection are minimized with the closed system. A concern pertaining to the closed gas measurement system is the build up of pressure in the flask which may hinder microbial digestion. Schofield and Pell (1995) demonstrated, that after a 48 h incubation, the build up of pressure does not adversely affect the extent or rate of digestion, indicating that 150 mg of substrate in a 50 ml Erlynmyer flask would be digested thoroughly by the ruminal microbes.

Gas measurement systems have been used to measure forage digestibility and the kinetics of microbial digestion (Pell and Schofield 1993, Theodorou et al., 1994, and Schofield
and Pell 1995). Two approaches that can be used to measure gas production \textit{in vitro} are gas collection at atmospheric pressure and its volume determined directly or the gas accumulation in a fixed volume container with gas volume determined through pressure changes (Menke et al., 1979 and Schofield et al., 1994). A computerized gas measurement system electronically records the changes in pressure in the fixed volume containers through the use of pressure transducers connected to fermentation flasks which are monitored by a data logger and computer interface (Pell and Schofield 1993 and Theodorou et al., 1994). The computerized method minimizes potential leakage problems and decreases human error associated with manual recording of gas volumes (Pell and Schofield 1993 and Schofield and Pell 1995). Accumulated gas measurement through pressure changes is a technique that has been shown to be a valid means of gas collection in comparison to the atmospheric methods (Pell and Schofield 1993 and Schofield and Pell 1994).

\section*{2.3 MATERIALS AND METHODS}

\subsection*{2.3.1 Viability of Yeast Culture}

A viable yeast cell count was conducted according to the Interpretation Guide for Petrifilm Yeast and Mold Count Plate (3M Microbiology Products, St.Paul, MN). A 25 g sample of dried yeast product was added to 225 ml of 0.1\% sterile peptone solution. The mixture was blended by vigorously shaking the mixture in a sealed 500 ml jar for 5 minutes. This mixture was then allowed to rehydrate for 30 minutes. Rehydration enabled all cells to be active but not to multiply. After the thirty minute period the mixture was blended again by shaking for 5 minutes. It was from that mixture that further dilutions were taken. Diluted suspensions of $10^{-3}$ consisted of one ml of the mixture added into 99 ml of 0.1\% peptone solution, repeated until a serial dilution of $10^{-5}$ was reached. Further diluted suspensions were made with one ml of the
$10^{-5}$ solution added into 9 ml of 0.1% peptone solution, repeated until a dilution of $10^{-9}$ was reached. Using petrifilm plates, supplied by 3M Microbiology Products for yeast and mold, duplicate plates were inoculated with one ml of each diluted sample starting at $10^{-5}$ through $10^{-9}$. Samples were incubated at 20-25°C (room temperature) for 5 days. On day 5, the plates were checked and colony forming units (cfu), which represented one colony, were counted. Yeast colonies were small and tan with well defined edges and no foci. It was determined that CYC contained live yeast cells at a population of $1\times10^9$ cfu/g of yeast preparation.

2.3.3 Treatments

There were seven treatments in the in vitro trial. The level of live yeast culture inclusion varied from 0.0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 % DM basis of the substrate (either barley grain or orchardgrass hay). All levels of inclusion were tested in quadruplicate.

2.3.4. Samples and Yeast Addition

One gram of dried ground barley grain or orchardgrass hay was weighed into a 50 ml beaker. The appropriate level of live yeast culture (0.0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 % DM) was added to the beaker based on the 1 g of substrate. Contents of beakers were mixed well to ensure even distribution of yeast in the sample. Samples of 150 mg were then removed from these beakers and weighed into modified 50 ml Erlynmyer flasks.

2.3.5 Animals and Feeding

Two rumen cannulated donor cows were used from South Campus, UBC, as the rumen inoculum source. Cows were fed a barley grain and orchardgrass hay diet in a ratio of 60:40, respectively. The barley grain was supplemented with 0.2% LYC on a dry matter basis. LYC was added to the grain using a feed mixer. The complete diet of the donor cows was 0.12 %
LYC on a dry matter basis. Cows were allowed a minimum of seven days to adapt to their new diet.

### 2.3.6 Rumen Fluid Collection

Rumen fluid collection occurred two hours post feeding from the dorsal region of the rumen. One liter of rumen contents was collected from each donor cow. One liter containers were filled to the top with rumen contents and sealed and placed in a bucket of warm water (39°C) for transport to the laboratory. On arrival at the laboratory the rumen fluid was strained through four layers of cheese cloth and glass wool. Rumen contents from both cows were then mixed together to reduce cow to cow variations. Processed fluid was allowed to settle for 20 min in a 4 L flask in an incubator set at 39°C. Rumen fluid was suctioned from the top of the 4 L flask. Warmed CO₂ was continuously bubbled through the suctioned rumen fluid which was then used as an inoculum source for subsequent *in vitro* gas measurement procedures.

### 2.3.7 Gas Measurement Modifications

Gas production measurements were made using a computerized, modified version of the procedures developed by Pell and Schofield (1993). Modifications consisted of utilizing a laboratory incubator that could maintain temperature at 39°C and contain a shaker plate which allowed for continuous orbital mixing at a set rpm of 100. A styrofoam holder was fastened to the shaker plate to produce a secure base for the insertion of the 50 ml Erlynmyer flasks. Flasks were inserted into the holder in four rows of eight which resulted in 32 flasks per run. Each flask was modified to have a 0.5 cm hole, in which stoppers that contained pressure transducers (PX170 series Omega Engineering, Inc., Stamford, CT, U.S.A.) were inserted. Each transducer was connected to a desktop computer through wiring called a channel. Small “ears” on the neck of the flask allowed for the attachment of small elastic bands which held the pressure transducer
and stopper in place. A small port on the side of the neck of the flask was fitted with a
Vacutainer stopper which allowed for rumen fluid injection, VFA sampling and system
calibration.

2.3.8 Procedures for Gas Measurement

Premade phosphate-bicarbonate buffer solution (13 ml) containing reducing solution was
prewarmed to 39°C and added to each of the 32 flasks that contained preweighed samples. The
composition of the buffer solution can be found in Appendix 1.0. Flasks were bubbled with
warm CO₂ for 15 seconds to establish an anaerobic environment. Stoppers that contained
pressure sensors were immediately moistened with distilled water and inserted into the mouth of
the flask. Stoppers were held in place by elastic bands. The flasks remained in the warm
incubator for one hour until the indicator in the buffer solution, resazurin, turned clear which
indicated an anaerobic environment. Composition of the resazurin solution can be found in
Appendix 1.0. Processed rumen fluid (3ml) was then injected through the septa arm into each
flask via a 10 ml Becton Dickinson disposable syringe with a 18 gauge needle.

2.3.9 Collection of Gas Data

Each run of the gas measurement system contained 32 flasks in total. Each level of LYC
inclusion had four replicates per incubation and two incubation trials were run for each
substrate. In addition to the 28 substrate flasks there were four blank flasks which contained
only buffer solution and processed rumen fluid. These blanks were averaged and used to
correct the 28 channels for rumen fluid activity. Gas measurements were made using pressure
transducers with a range of 0 to 103 kPa (0 to 15 psi). These transducers measured the pressure
within the flask, which resulted from gas production by rumen micro-organisms. The pressure
measured generates a differential voltage output proportional to that pressure. The output
voltage was measured using Tempscan/1000 (Omega Engineering, Inc., Stamford, CT, U.S.A.). Tempscan/1000 is a high speed data logger system set to acquire data from the 32 channels with the ability to transfer the data to a computer into Tempwindows 3.1. Channels were set to take pressure readings every 10 minutes over a 24 h incubation period.

2.3.10 Calibration of Gas Measurement Machine

Calibration is required because of the pressure related changes in CO₂ solubility (Schofield and Pell 1995). This calibration of the sensors enables the determination of milliliters of gas needed to produce a 1 millivolt change in sensor output (Schofield and Pell 1995). The accurate calibration of the gas measurement machine is important in order to be able to obtain a linear relationship between the amount of gas produced and the voltage response of the pressure sensors. The calibration of the gas measurement machine was conducted in a similar manner as the procedures outlined in section 2.3.8. The differences were that only five erlenmyer flasks were used instead of all 32 and that no rumen fluid inoculum was used. Instead 15 ml of buffer solution warmed to 39°C was injected into all 5 flasks. The composition of the buffer solution can be seen in Appendix 1.0. Flasks were sequentially injected with CO₂ through the septa and allowed to equilibrate. The zero voltage reading was taken and then 5 ml of CO₂ was injected into each of the 5 flasks. These flasks were allowed to equilibrate for ten minutes and then the voltage reading was taken. This method was repeated for the subsequent injection of 10, 15 and 20 ml of CO₂. From the average voltage readings taken at each CO₂ level, a straight line was determined through regression analysis. This regression output can be seen in Appendix 2.0. A regression equation was developed to describe the relationship and to convert voltage response into resulting gas volume, ml (Pell and Schofield 1993). Sensor responses were obtained by subtraction of the difference between the voltage recorded at baseline from the voltage recorded at 10 minutes.
\[ Y = mX + b, \]  
(Equation 1)

where \( Y \) is the ml of \( CO_2 \), \( m \) is the slope of the line, \( X \) is the voltage response and \( b \) is the y-intercept.

Voltage responses from the channels were converted to gas volumes using the following regression equation:

\[ Y = \frac{72.7718 \text{ ml}}{VX} - 43.125 \text{ ml}, \]  
(Equation 2)

where \( Y \) is the gas volume and \( X \) is the voltage response. The R squared value for this equation is 0.97.

2.3.11 Analysis of Gas Data

Voltage responses are subjected to a series of conversions in order to determine the gas production volumes. Firstly, the baseline voltage at time zero was subtracted from the observed voltage at any time for that channel. This must be conducted for all channels individually. Secondly, there is the conversion of the net voltage output to gas volume using the pressure sensor calibration correction factor (Equation 2). Thirdly, subtract the gas volume produced from the blank flasks. Finally, the normalization of the net gas production volume to the standard 1 g dry sample. The gas production data was then fitted to simple limited exponential growth (SLEG) model with lag. This model was used for \textit{in vitro} gas measurement by Krishnamoorthy et al., 1991, 1995.

\[ Y = A(1 - e^{-b(t-1)}), \]  
(Equation 3)

where \( Y \) is the cumulative gas production at a given time \( t \), \( l \) is the lag of fermentation and \( A \) is the potential gas production which may be produced at a specific rate \( b \) (h\(^{-1}\)) determined through non-linear regression analysis.
2.3.12 Volatile Fatty Acid Analysis

Samples containing rumen fluid and buffer solution were taken from the incubation flasks at 0 h and 24 h incubation times for all level of LYC inclusion. Samples were stored in sealed 2 ml micro centrifuge tubes and frozen to – 20°C for further analysis. Volatile fatty acid (VFA) determination was conducted by using a Shimadzu gas chromatograph equipped with a capillary column (30 m x 0.25 mm I.D. Stabilwax-DA). Injection port temperature was set at 170°C. The column temperature was set at 120 to 180°C at 10°C min⁻¹, with an initial time of one minute and a final time of two minutes. A flame detector (FID) was used at a temperature of 190°C with a carrier gas of helium. Sample injection amount was 1 ul. The flow rate of the gases 5 to 10 ml/min and 40 to 80 ml/min. The internal standard used was isocaproic acid (0.70 g in 200 ml water).

2.3.13 Statistical Analysis

Statistical analysis was conducted using the General Linear Model (GLM) procedure of SAS (SAS 1990). Statistically significant differences (P<0.05) were determined using the least square means for cummulative gas production, potential gas production, rate of gas production, length of lag phase, total VFA production and acetate:propionate ratios.

\[ Y_{ij} = u + T_i + P_j + e_{ij} \]

\( u \) = overall mean

\( T_i \) = effect of treatment, \( i = 1, \ldots, 7 \)

\( P_j \) = effect of rep, \( j = 1, 2 \)

\( e_{ij} \) = experimental error
2.4 RESULTS

2.4.1. Cumulative Gas Production

Cumulative gas is the total amount of gas produced during the incubation period of 24 h. There were no significant (P>0.05) differences between any of treatments for the cumulative gas production for barley grain (Table 2.1). For orchardgrass hay there were no significant (P>0.05) differences between any of the LYC treatments for cumulative gas production (Table 2.2). Despite the lack of significant (P>0.05) increases in gas production there was a numerical trend towards increased cumulative gas production with both substrates as the level of LYC inclusion increased.

2.4.2 Potential Gas Production

Potential gas production is the amount of gas that could be potentially produced from the substrate if the incubation was to continue to completion. For barley grain all levels of the LYC inclusion, except 0.10 % LYC DM, produced a significantly (P<0.05) higher potential gas production when compared to the control (Table 2.1). There were significantly (P<0.05) higher levels of potential gas production (15.80, 16.89 and 15.59 mg/ml DM) at the 0.20, 0.25 and 0.30 % LYC inclusion level respectively when compared to other levels of LYC inclusion (Table 2.1). The addition of 0.10 % LYC resulted in a significantly (P<0.05) lower potential gas production than observed for the control (Table 2.1). For orchardgrass hay, all levels of LYC inclusion resulted in significantly (P<0.05) higher potential gas production when compared to the control. The highest potential gas productions (16.09 and 16.75 mg/ml DM) were seen at the 0.2 and 0.3% LYC inclusion levels respectively (Table 2.2).
2.4.3 Rate of Gas Production

Rate of gas production is the rate at which gas was produced in the incubation vessel. There was a significant (P<0.05) decrease in the rate of gas production at the 0.2 and 0.25% LYC inclusion levels (0.129 and 0.106 ml/h), compared to the control (0.206 ml/h) (Table 2.1). Inclusion of LYC at levels of 0.05, 0.10 and 0.15 increased (P<0.05) the rate of gas production relative to the control sample (Table 2.1). With orchardgrass hay a fast rate of gas production in the rumen is ideal because this represents a fast rate of fermentation in the rumen, thus more forage will be digested. In the current study all levels of LYC showed a significantly (P<0.05) slower rate of gas production when compared to the control (Table 2.2). The fastest rate of gas production was produced both by the control, 0.0 % LYC, (0.12 ml/h) and the 0.25 % LYC (0.118 ml/h) inclusion level (Table 2.2).

2.4.4 Lag of Fermentation

The lag of fermentation is the time required for the hydration of the feed particles, fermentation of soluble sugars and colonization and attachment of rumen microbes to feed particles. A short or decreased lag phase represents the ease with which these processes occur. All levels of LYC, except the LYC level of 0.05 %, significantly (P<0.05) decreased the lag of fermentation for barley grain when compared to the control. Addition of 0.05 % LYC resulted in an increase (P<0.05) in the lag time relative to the control (Table 2.1). A significant (P<0.05) decrease in lag time (0.05 and 0.07 h) was seen at the 0.2 and 0.25% LYC inclusion levels compared to the control (0.47 h) (Table 2.1). A significant (P<0.05) decrease in lag of fermentation of orchardgrass hay was seen for all levels of LYC inclusion compared to the control, except for the 0.30 % LYC which was similar to the control (0.91 vs. 0.95 h respectively) (Table 2.2). The most dramatic decrease in lag was observed for the 0.2% LYC inclusion level (0.2 h) compared to the control (0.95 h) (Table 2.2).
2.4.5 Volatile Fatty Acids

With barley grain as a substrate there was a significant (P>0.05) difference between the 0 and 24 h sampling times, for all VFAs, except isobutyrate (Table 2.3). For orchardgrass hay all VFAs, except butyrate and caproate, were significantly (P<0.05) different for 0 and 24 h sampling times (Table 2.4). Both barley grain and orchardgrass hay had significantly (P<0.05) higher total VFA production at 24 h vs. 0 h sampling times (Table 2.3 and Table 2.5 respectively). Analysis of VFA production for all levels of LYC inclusion with barley grain showed that the total production was significantly (P<0.05) higher for the 0.1 % LYC level (389.3 mol) when compared to the control (0.0 % LYC) (264.6 mol) (Table 2.5). All other levels of LYC produced total VFA similar to that of the control (Table 2.5). Acetate, propionate and the acetate:propionate ratio for barley grain were not significantly (P>0.05) different for any level of LYC inclusion (Table 2.5). Inclusion of LYC significantly (P<0.05) affected the molar percentages of isobutyrate, butyrate, isovalerate, valerate, caproate and branched VFA (Table 2.5). The analysis of total VFA production from orchardgrass hay for all seven levels of LYC inclusion resulted in a lack of significance (P>0.05) for any inclusion level compared to the control (0.0 % LYC) (Table 2.6). For orchardgrass hay all levels of LYC produced acetate levels that were numerically lower than the control (Table 2.6). Propionate, isobutyrate, isovalerate, valerate and branched VFA's were not significantly (P>0.05) influenced by any LYC inclusion level when compared to the control (Table 2.6). The acetate:propionate ratio for the control was significantly (P<0.05) higher than all other levels of LYC (Table 2.6). The 0.5 % LYC inclusion level stimulated a significantly (P<0.05) higher molar percentage of butyrate production from orchardgrass hay than the control (Table 2.6). The 0.05 % LYC inclusion level produced a significantly (P<0.05) higher molar percentage of caproate compared to the control (Table 2.6).
2.6 DISCUSSION

The current study demonstrated that inclusion of LYC at varying levels in an *in vitro* system did not alter cumulative gas production from barley grain or orchardgrass hay. The absence of change in the cumulative gas production was also seen in a study by Rouzbehian et al., (1996) for either barley grain or orchardgrass hay. This is in contrast to other studies (Gray and Ryan 1987 and Mutsvangawa et al., 1992) that have noted an increase in the cumulative *in vitro* gas production from the addition of LYC to both barley grain and orchardgrass hay. Differences between the current study and studies of Gray and Ryan (1987) and Mutsvangwa et al., (1992) could be due to the time of rumen fluid collection. Menke and Steingass (1988) claim that rumen fluid for *in vitro* work should be collected prior to feeding so that the fluid has less activity and is more constant in composition (Rouzbehian et al., 1994). Other studies have shown that rumen fluid collection 2-4 h post feeding produced similar results to those studies that used inoculum collected prior to feeding (Windschitl 1992 and Kung et al., 1997). For the current study, the collection of rumen inoculum occurred 2 hs post feeding.

The potential gas that was produced from either barley grain or orchardgrass hay substrate was significantly (P<0.05) higher for all levels of LYC inclusion when compared to the control (Tables 2.1 and 2.2 respectively). Studies which used the *in vitro* gas measurement machine have been interested in the cumulative gas produced, rate of gas production or the lag of fermentation (Krishnamoorthy et al., 1991, Schofield et al., 1994, Theodorou et al., 1994 and Schofield and Pell 1995). No studies were found that mentioned the ability to measure the potential gas production from the substrate.

The rate of gas production for barley grain and orchardgrass hay was significantly (P<0.05) lower for all levels of LYC inclusion when compared to the control (Tables 2.1 and Table 2.2).
For barley grain, this result is in contrast to previous studies of Gray and Ryan (1987), Frumholtz et al., (1989) and Kung et al., (1997) who claimed that the rate of gas production from barley grain increases with addition of LYC in the *in vitro* system. Barley grain is more rapidly and extensively degraded in the rumen than hay (Windschitl 1992). Barley grain based concentrates have a more rapid rate of passage from the rumen than corn based concentrates or forages (Menke and Steingass 1988 and Frumholtz et al., 1989). Decreased rate of digestion of barley grain in the rumen would suggest that the readily digestible carbohydrate would be passed on to the small intestine for further digestion.

For orchardgrass hay a decrease in the rate of gas production is not as desirable as it would be for barley grain. The hay is degraded slowly in the rumen and remains there for some time, usually greater than 24 hours (Menke and Steingass 1988). An increased rate of gas production would indicate greater ruminal fermentation. A faster rate of gas production would indicate a greater digestion of forage in the rumen, which would suggest an increased rate of passage and thus an increased dry matter intake. In the current study the rate of gas production for orchardgrass hay was significantly (*P*<0.05) decreased with the inclusion of LYC. This would suggest that LYC did not improve the digestibility of the orchardgrass hay. It is possible that inclusion of LYC had no affect or actually decreased the digestibility of orchardgrass hay. Digestibility of the orchardgrass hay was not determined in the current study. Previous studies have noted increased feed efficiency and increased digestibility of forages (Adams et al., 1981, Wallace et al., 1981, Dawson and Newman 1987, Gray and Ryan 1987, Olson et al., 1994, and Newbold et al., 1995). Both studies of Gray and Ryan (1987) and Olson et al., (1994) claimed that the inclusion of LYC did not change the rate of gas production, while the other studies (Adams et al., 1981, Wallace et al., 1981, Dawson and Newman 1987, and Newbold et al., 1995) did not document the rate of gas production, so it is unknown if there were decreased rates of gas production similar to the current study.
The decreased rate of gas production coincides with the absence of increased total VFA production. If the orchardgrass hay was digested slower in the rumen less gas would be produced and with less VFAs produced. In the current study there was a trend towards increased total VFA production as the LYC level increased but there was no significant (P>0.05) difference between the control (0 % LYC) and any level of LYC inclusion. Inclusion of LYC had no significant (P>0.05) effect on the cumulative gas production, but there was a trend towards increased cumulative gas production as the level of LYC inclusion increased. This would indicate that inclusion of LYC in a orchardgrass hay based ration has the potential to increase the cumulative gas production and total VFA production, although this was not demonstrated in the current study.

The length of the lag of fermentation was significantly (P<0.05) decreased for both the barley grain and the orchardgrass hay. The most dramatic drop in lag was seen at the 0.2 % LYC inclusion level for both the barley grain and the orchardgrass hay. For barley grain, the lag time decreased from 0.47 h (28 min) for the control, 0.0 % LYC, to 0.06 h (3.36 min) for the 0.2 % LYC level, a decrease of 25 minutes (Table 2.1). This suggests that the microbes in the rumen began digesting the barley grain more quickly with the inclusion of LYC.

For orchardgrass hay the lag time dropped from 0.95 h (57 min) for the control, 0 % LYC, to 0.20 h (12 min) for the 0.2% LYC level (Table 2.2). This suggests, as for the barley grain, that the inclusion of LYC stimulated the microbes in the rumen to begin digesting the feed particles more quickly. Since the onset of fermentation was decreased this would suggest that more forage would be able to be digested in the rumen. These results are comparable to in vitro work by Williams et al., (1991) and Olson et al., (1994) in which the inclusion of LYC decreased the lag time of hay digestion.

Inclusion of LYC in the incubation of barley grain produced a trend towards higher total VFA production with increasing LYC inclusion level. Analysis of the individual VFAs
indicated that the acetate and propionate production levels were unaffected by the inclusion of LYC. This is similar to a previous study by Dawson (1993) that noted the total VFA production can be altered with LYC inclusion while the individual VFA proportions remain unchanged.

For orchardgrass hay LYC had no significant (P>0.05) influence on total VFA production. This result is similar to other in vitro gas measurement studies that have utilized LYC supplementation (Harrision et al., 1988, Piva et al., 1989, Dawson 1990, Carro et al., 1992, Rouzbehan et al., 1994, and Schofield and Pell 1995). Lack of a change in total VFA production suggests that the end products of fermentation were unaffected by the inclusion of LYC. Analysis of individual VFA's indicated that there was a decrease in acetate and an increase in propionate production with LYC inclusion. This is similar to other studies that indicated the individual levels of VFA's could change with the LYC inclusion but the total VFA production remained unchanged (Gray and Ryan 1987, Carro et al., 1992, and Mustvanagwa 1992). Results are comparable to other studies of Adams et al., (1981), Harrison et al., (1988), Dawson et al., (1990), and Newbold et al., (1990) that claimed LYC inclusion will cause an increase in propionate at the expense of acetate. Other in vitro studies noted production of higher levels of acetate and lower levels of propionate with inclusion of LYC, without altering total VFA production (Gray and Ryan 1987, Carro et al., 1992, Kumar et al., 1992, Mustvanagwa 1992, and Zelenak et al., 1994). No clear pattern of LYC effect on VFA production has been established. These variable responses suggest that the changes in VFA production are the result of an effect of LYC on the microbial population rather than on VFA production (Newbold et al., 1998).

The patterns of ruminal fermentation may not have been dramatically altered by the inclusion of LYC but both the rate and lag of fermentation significantly (P<0.05) benefited from LYC inclusion. The improved potential gas production and decreased lag of fermentation seen
with the inclusion of LYC for both barley grain and orchardgrass hay could be explained by an increase in numbers of microbes in the rumen. In the current study the numbers of bacteria in the rumen fluid were not enumerated but a review of the literature suggests that increased numbers in the rumen are the main factor responsible for the changes in fermentation patterns (Newbold et al., 1987, Weidmeier et al., 1987, Dawson 1990, Newman et al., 1990, Piva et al., 1993, Newbold et al., 1995, and Kung et al., 1997). It has been proposed by Rose (1987), Piva et al., (1989), and Dawson 1990, and Newman et al., (1990), that the LYC may provide factors such as nucleotides, amino acids, vitamins, growth factors and enzymes (Piva et al., 1989), that stimulate the growth of bacteria in the rumen.

2.7 CONCLUSION

Considering all four parameters of gas production; cummulative, potential, rate of gas production and the lag of fermentation it can be concluded that the level of 0.2% DM LYC for both the barley grain and orchardgrass hay had the most potential to elicit responses in dairy cows. This was also the recommended level of LYC inclusion of the manufacturer of the Live Yeast Culture product, Choong Ang Chemical Company Ltd.
2.8 REFERENCES


Kellner, R.J. and M. Kirchhgessner. 1976. Evaluation of the net energy of green feed and roughage from their crude nutrient content and digestibility estimated by cellulase


Table 2.1 Effect of LYC supplementation on gas measurement parameters of barley grain.

<table>
<thead>
<tr>
<th>Gas Parameter</th>
<th>LYC inclusion level (% DM)</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cummulative gas production (ml/mg DM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0*</td>
<td>12.472</td>
<td>13.346</td>
<td>11.583</td>
</tr>
<tr>
<td>Potential gas production (ml/mg DM)</td>
<td>12.897b</td>
<td>13.750c</td>
<td>12.244a</td>
</tr>
<tr>
<td>Rate of gas production (ml/h)</td>
<td>0.206d</td>
<td>0.214e</td>
<td>0.220e</td>
</tr>
<tr>
<td>Lag of fermentation (h)</td>
<td>0.471d</td>
<td>0.625e</td>
<td>0.417c</td>
</tr>
</tbody>
</table>

a, b, c, d, e, f Means in the same row with different letters differ significantly (P<0.05).
* 0.0 % LYC DM basis is the control
Table 2.2 Effect of LYC supplementation on gas measurement parameters of orchardgrass hay.

<table>
<thead>
<tr>
<th>Gas Parameter</th>
<th>LYC inclusion level (% DM)</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0*</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>Cummulative gas production</td>
<td>10.797</td>
<td>12.995</td>
<td>11.172</td>
</tr>
<tr>
<td>(ml/mg DM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potential gas production</td>
<td>11.876a</td>
<td>15.131e</td>
<td>12.714b</td>
</tr>
<tr>
<td>(ml/mg DM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate of gas production</td>
<td>0.121f</td>
<td>0.096c</td>
<td>0.103d</td>
</tr>
<tr>
<td>(ml/h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag of fermentation</td>
<td>0.953d</td>
<td>0.564c</td>
<td>0.351b</td>
</tr>
<tr>
<td>(h)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a, b, c, d, e, f, g Means in the same row with different letters differ significantly (P<0.05).

* 0.0 % LYC DM basis is the control.
Table 2.3  Effect of all levels of LYC on 0 h and 24 h volatile fatty acid production from barley grain.

<table>
<thead>
<tr>
<th>Volatile Fatty Acid (mol/ 100 mol)</th>
<th>0 h</th>
<th>24 h</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetate</td>
<td>66.11b</td>
<td>53.61a</td>
<td>+/- 1.16</td>
<td>0.0001</td>
</tr>
<tr>
<td>propionate</td>
<td>13.60a</td>
<td>23.18b</td>
<td>+/- 1.16</td>
<td>0.0001</td>
</tr>
<tr>
<td>isobutyrate</td>
<td>0.91</td>
<td>1.58</td>
<td>+/- 0.27</td>
<td>0.0948</td>
</tr>
<tr>
<td>butyrate</td>
<td>17.16a</td>
<td>18.6b</td>
<td>+/- 0.37</td>
<td>0.0108</td>
</tr>
<tr>
<td>isovalerate</td>
<td>0.95a</td>
<td>1.36b</td>
<td>+/- 0.09</td>
<td>0.0044</td>
</tr>
<tr>
<td>valerate</td>
<td>0.89a</td>
<td>1.44b</td>
<td>+/- 0.18</td>
<td>0.0439</td>
</tr>
<tr>
<td>caproate</td>
<td>0.37b</td>
<td>0.24a</td>
<td>+/- 0.03</td>
<td>0.0020</td>
</tr>
<tr>
<td>a: p ratio¹</td>
<td>4.94b</td>
<td>2.53a</td>
<td>+/- 0.19</td>
<td>0.0001</td>
</tr>
<tr>
<td>branched²</td>
<td>1.86a</td>
<td>2.93b</td>
<td>+/- 0.30</td>
<td>0.0127</td>
</tr>
<tr>
<td>total (mols)</td>
<td>155.71a</td>
<td>437.66b</td>
<td>+/-17.00</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

a, b Means in the same row with different letters differ significantly (P<0.05).

¹ acetate:propionate ratio
² branched consists of the sum of isobutyrate and isovalerate
Table 2.4  Effect of all levels of LYC on 0 h and 24 h volatile fatty acid production from orchardgrass hay.

<table>
<thead>
<tr>
<th>Volatile Fatty Acid (mol / 100mol)</th>
<th>0 h</th>
<th>24 h</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetate</td>
<td>66.26a</td>
<td>60.18b</td>
<td>+/- 0.88</td>
<td>0.0001</td>
</tr>
<tr>
<td>propionate</td>
<td>15.10a</td>
<td>20.45b</td>
<td>+/- 0.64</td>
<td>0.0001</td>
</tr>
<tr>
<td>isobutyrate</td>
<td>1.07a</td>
<td>1.39b</td>
<td>+/- 0.06</td>
<td>0.0005</td>
</tr>
<tr>
<td>butyrate</td>
<td>14.53</td>
<td>13.19</td>
<td>+/- 0.92</td>
<td>0.3081</td>
</tr>
<tr>
<td>isovalerate</td>
<td>1.44a</td>
<td>2.33b</td>
<td>+/- 0.10</td>
<td>0.0001</td>
</tr>
<tr>
<td>valerate</td>
<td>1.20a</td>
<td>2.08b</td>
<td>+/- 0.13</td>
<td>0.0001</td>
</tr>
<tr>
<td>caproate</td>
<td>0.40</td>
<td>0.39</td>
<td>+/- 0.02</td>
<td>0.7961</td>
</tr>
<tr>
<td>a:p ratio¹</td>
<td>4.40a</td>
<td>3.07b</td>
<td>+/- 0.21</td>
<td>0.0002</td>
</tr>
<tr>
<td>branched²</td>
<td>2.66a</td>
<td>3.71b</td>
<td>+/- 0.17</td>
<td>0.0003</td>
</tr>
<tr>
<td>total (mols)</td>
<td>148.48a</td>
<td>323.04b</td>
<td>+/-16.00</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

a,b Means in the same row with different letters differ significantly (P<0.05).
¹ acetate:propionate ratio
² branched consists of the sum of isobutyrate and isovalerate
Table 2.5  Effect of LYC on volatile fatty acid production for all levels of LYC from barley grain.

<table>
<thead>
<tr>
<th>Volatile Fatty Acid (mol/ 100 mol)</th>
<th>Levels of LYC inclusion (% DM)</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>acetate</td>
<td>60.62</td>
<td>58.74</td>
<td>59.86</td>
</tr>
<tr>
<td>propionate</td>
<td>18.22</td>
<td>19.63</td>
<td>15.52</td>
</tr>
<tr>
<td>isobutyrate</td>
<td>0.92a</td>
<td>0.67a</td>
<td>3.36a</td>
</tr>
<tr>
<td>butyrate</td>
<td>17.41ab</td>
<td>18.44ab</td>
<td>19.16ab</td>
</tr>
<tr>
<td>isovalerate</td>
<td>1.32ab</td>
<td>1.37ab</td>
<td>0.99ab</td>
</tr>
<tr>
<td>valerate</td>
<td>1.15ab</td>
<td>0.89a</td>
<td>0.79a</td>
</tr>
<tr>
<td>caproate</td>
<td>0.36ab</td>
<td>0.26a</td>
<td>0.30ab</td>
</tr>
<tr>
<td>a: p ratio(^1)</td>
<td>3.54</td>
<td>3.47</td>
<td>3.91</td>
</tr>
<tr>
<td>branched(^2)</td>
<td>2.24a</td>
<td>2.04a</td>
<td>4.36b</td>
</tr>
<tr>
<td>total (mols)</td>
<td>264.6a</td>
<td>281.7a</td>
<td>389.3b</td>
</tr>
</tbody>
</table>

\(^a,b\) Means in the same row with different letters differ significantly (P<0.05).
\(^1\) acetate:propionate ratio
\(^2\) branched consists of the sum of isobutyrate and isovalerate
Table 2.6 Effect of LYC on volatile fatty acid production for all levels of LYC from orchardgrass hay.

<table>
<thead>
<tr>
<th>Volatile Fatty Acid (mol/100 mol)</th>
<th>Levels of LYC inclusion (% DM)</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>acetate</td>
<td>67.98c</td>
<td>62.15ab</td>
<td>61.07ab</td>
</tr>
<tr>
<td>propionate</td>
<td>15.96</td>
<td>17.27</td>
<td>18.76</td>
</tr>
<tr>
<td>isobutyrate</td>
<td>1.16</td>
<td>1.20</td>
<td>1.17</td>
</tr>
<tr>
<td>butyrate</td>
<td>11.56a</td>
<td>15.27ab</td>
<td>15.31ab</td>
</tr>
<tr>
<td>isovalerate</td>
<td>1.58</td>
<td>1.87</td>
<td>1.83</td>
</tr>
<tr>
<td>valerate</td>
<td>1.41</td>
<td>1.74</td>
<td>1.49</td>
</tr>
<tr>
<td>caproate</td>
<td>0.34a</td>
<td>0.49b</td>
<td>0.37ab</td>
</tr>
<tr>
<td>a: p ratio&lt;sup&gt;1&lt;/sup&gt;</td>
<td>4.61b</td>
<td>3.72ab</td>
<td>3.43a</td>
</tr>
<tr>
<td>branched&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.17</td>
<td>3.07</td>
<td>3.02</td>
</tr>
<tr>
<td>total (mols)</td>
<td>206.3</td>
<td>237.1</td>
<td>247.3</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means in the same row with different letters differ significantly (P<0.05).

<sup>1</sup> acetate:propionate ratio

<sup>2</sup> branched consists of the sum of isobutyrate and isovalerate
3.0 EFFECT OF LIVE YEAST CULTURE SUPPLEMENTATION ON DRY MATTER INTAKE AND MILK PRODUCTION OF EARLY PRODUCTION COWS

3.1 ABSTRACT

Decreases in feed intake of 30 – 35 % are not uncommon 2 – 3 days prior to parturition. Failure to meet the increased requirements for nutrients particularly energy and minerals could result in a range of post partum health problems that may compromise lactational performance. It has been shown that the feeding live yeast cultures (LYC), *Saccharomyces cerevisiae*, to cows in early lactation may increase both dry matter intake (DMI) and milk production. The objective of this study was to determine the effect of LYC supplementation on DMI and milk yield of early lactation dairy cows. Twenty-four multiparous cows were paired, based on age, body weight and previous 305 d milk production, and randomly assigned to one of two treatment groups, control or yeast supplemented. Cows were fed a basal ration twice daily consisting of 85 % forage and 15 % concentrate on a dry matter (DM) basis. The LYC was fed at a level of 0.2 % of estimated DMI. All cows received 1.0 kg of concentrate fed separately from the basal ration twice daily. Yeast allotment was divided into two equal portions and hand mixed with the separately fed concentrate. The supplementation of LYC began 7 d prior to expected calving date, this was referred to as the pre partum period. The lactation trial was divided into two experimental periods: period one which lasted from 0 – 14 DIM, and period two which lasted from 15 – 85 DIM. LYC supplementation was continued in period one but stopped as cows entered period two. Daily DMI, milk yield, body condition score (BCS), body weight (BW) and selected blood parameters were recorded in period one. The concentrate allotment was increased by increments of 0.5 kg per day post calving, until completion of period one. Rumen and blood samples were taken on days seven and fourteen of period one. Milk production was recorded daily for period one and period two. During the 7 d pre partum
period the inclusion of LYC had no significant (P>0.05) effect on DMI between the control and LYC supplemented groups. Results indicate that there was no significant (P>0.05) difference in terms of either DMI or average milk yield for period one. Analysis of individual daily milk yields indicated a trend towards higher milk production with LYC supplementation which reached significance (P<0.05) on day 14 of period one. Period two showed a significant (P<0.05) increase in milk production of an average 3.73 kg/d for cows previously supplemented with dietary LYC. The previously supplemented cows showed higher and longer peaks of lactation, indicating that LYC supplementation in the transition period was beneficial for early lactation.

3.2 INTRODUCTION

The transition from late pregnancy to early lactation is a time period in which dairy cows are under considerable amounts of stress from rapid fetal growth and mammary development (Grummer 1995). Nutritional requirements, during this period, increase by 30 – 50% (Bertics et al., 1992). The ability of a cow to meet these requirements is compromised by a decrease in DMI by 30 – 40% in the pre partum period. This decrease in DMI often leads to a negative energy balance affecting subsequent health, lactational and reproductive performance (Erb et al., 1985, Bertics et al., 1992, Pedron et al., 1993, Grummer 1995, and Robinson 1997). The exact cause of the drop in DMI is not known but it is speculated that it is hormonally controlled by changes in estrogen levels (Journet and Redmond 1976, and Bertics et al., 1992). Benefits of increasing DMI around parturition are a decrease in the incidence of post partum health problems and enhancement of early lactational performance. Supplementation of cows during this transition period with LYC may have beneficial effects by stimulating an increase in the microbial population with a subsequent increase in DMI and increased milk production.
Lack of scientific studies examining the nutritional requirements and management strategies of pre partum and early lactation dairy cows has encouraged interest in this area. Feeding and management of pre partum dairy cows is often extrapolated from the research of dairy cows in other stages of lactation, although the needs of a early lactating dairy cow are different from that of a high producing dairy cow. It is plausible that the knowledge acquired from lactating dairy cows could be applied to pre partum and early lactation cows without compromising their health, but it is important to look at the transition from late pregnancy to early lactation separately from other stages of lactation.

Over the years through extensive research with lactating dairy cattle, the benefits of feed additives such as live yeast culture (LYC) have been documented (Wohlt et al., 1991, Erasmus et al., 1992, Dawson 1993, and Stewart 1995). Information has been collected as to the numerous proposed modes of action of LYC in the rumen and the conditions which offer the best responses from inclusion. Specific responses such as increased DMI, VFA profile changes, increased milk yield and stabilization of the rumen environment are desirable in the transition dairy cow. Management of pre partum dairy cows is important in order to maximize early milk production and DMI. While a decrease in DMI during this period is probably inevitable, it would be beneficial to reduce the magnitude and duration of this decrease.

Previous studies (Erdman 1988, Wohlt et al., 1991, Robinson 1997, and Wohlt et al., 1998) conducted on pre partum cows have indicated that LYC may be more beneficial during this period than in later lactation. Reasoning for this belief is that cows are in a negative energy balance at this time with dry matter intake (DMI) decreased by up to 40% (Kertz et al., 1991) and DMI being a major factor limiting milk production in early lactation. Decreased DMI leads to loss of BW, loss of body condition score, loss in milk production, all because energy (nutrient) requirements cannot be met by daily ration intake. Cows utilize fat reserves and are therefore more susceptible to metabolic disorders such as ketosis and milk fever. Yeast
inclusion in the ration of pre partum and early lactation cows may be beneficial as demonstrated by increased milk production and increased dry matter intake before and after calving. The objective of the current study was to determine the effect of LYC supplementation on the dry matter intake and milk yield of production in early lactation dairy cows.

3.3 MATERIALS AND METHODS

3.3.1 Experimental Design

An *in vivo* lactation trial was conducted at the UBC Dairy Education and Research Center in Agassiz B.C. during the months from February to August 1997. Thirty multiparous Holstein cows were assigned to one of two groups, control or yeast supplemented. Groups were balanced according to age, body weight, lactation number and previous 305 d lactation. Six cows were removed from the trial at varying stages of the lactation trial. Cows 8942 and 91021, control cows, were removed during the trial due to milk fever and chronic mastitis respectively. Subsequently cows 9013 and 9021 were removed from data after the trial was completed as they were the LYC supplemented cows paired with the cows 8942 and 91021 that were removed due to illness. The data from cow pair 9337 and 90024 was deleted from the study because the LYC supplemented cow was predisposed to high milk yield. The trial began 7 d prior to expected parturition, this is referred to as the pre partum period. The lactation trial was divided into two distinct experimental periods: period one, 0 – 14 DIM, in which dry matter intake, milk yield, milk composition, specific blood parameters, rumen pH and rumen volatile fatty acids were measured for both control and yeast supplemented cows and period two, 15 – 85 DIM, milk yield and milk composition were recorded. LYC was supplemented during both the pre partum and period one of this study. Cows were weighed for three consecutive days
prior to entering the pre partum period and after leaving period one. Body condition scores were recorded upon entering period one and at seven and fourteen days post partum. Cows were individually penned, with only six cows in the pre partum or period one at any one time. For period two cows were housed in a free stall barn with the high producing group of the main dairy herd.

3.3.2 Yeast Allotment

Estimated dry matter intake of 2% of body weight was calculated based on the average of three consecutive day pre trial weights taken for each cow. Yeast allotment was assigned at the level of 0.2% of the estimated DMI. The 0.2% level of yeast inclusion was selected from the previous in vitro laboratory work using the modified Menke et al., (1979) gas measurement system at UBC (Chapter 2). Yeast was pre-measured into two equal portions per cow per day and stored at 5°C until fed with a concentrate premix. The level of yeast fed per cow was maintained at 0.2% of the estimated DMI throughout the LYC supplementation period as calculated for each cow at the beginning of period one.

3.3.3 Diet

Cows were fed a basal ration consisting of 85% forage and 15% concentrate (6:1 ratio forage to concentrate respectively) on a dry matter (DM) basis fed twice daily at 8:00 A.M. and 3:00 P.M. Cows were allowed free access to water and ad libitum access to feed. Weighbacks of the concentrate, hay and basal ration components were recorded each morning of the pre partum period and period one. In addition to the basal ration, all cows received 2 kg (as fed) of hay once daily and 1 kg (as fed) of concentrate twice daily. Both hay and concentrate were fed separately from the basal ration. Yeast allotment was divided into two equal portions and hand mixed into this separately fed concentrate for those cows receiving yeast supplementation. This
was to ensure that all cows received the same diet and that the yeast supplemented cows received their full yeast allotment. In period one the separately fed concentrate was increased by increments of 0.5 kg/d, for all cows until 14 DIM at which time they were transferred to a corn silage and barley based concentrate, 50:50 ratio, ration offered to the main herd.

3.3.4 Medical Treatments

Animals were cared for according to the standards of care as per Canadian Council on Animal Care (CCAC 1993). When animals on the trial went off feed they were examined and treated as required. During the trial standard farm practices and recommendations of the veterinarian were applied to maintain the health of the animals. Health problems encountered were ketosis, milk fever, acidosis and mastitis. Treatment for ketosis and acidosis was a ketoroid drench, containing choline chloride, potassium iodide, cobalt sulfate and propylene glycol (Austin, division of Vetoquinol Canada inc., St-Pierre Sud, Joliette, QU). Milk fever cows were given calcium raboroglucose 23 % (Austin, division of Vetoquinol Canada inc., St-Pierre Sud, Joliette, QU) subcutaneous and intravenously administered Calplus, containing calcium, magnesium, phosphorus and dextrose (MTC Pharmaceuticals, Cambridge, ON). Mastitis was treated by Ceflak, containing penicillin G Procaine (The Upjohn Company – Animal Health Division. Orangeville, ON) and 35 – 40 cc of Trivetrin, containing trimethoprim and sulfadoxine (Schering-Plough, a division of Schering Canada Inc., Pointe-Claire, QU).

3.3.5 Feed Sampling

All sampling of feed, weighbacks and hay were conducted each morning of the pre partum period and period one. The basal ration was sampled three times weekly, composited for that week and frozen to negative 14°C. Weighbacks were sampled twice weekly, composited for that week and frozen. Hay, corn silage, grass silage and concentrate samples were sampled
once weekly and frozen. Dry matter content was determined by drying the samples in a forced air oven for 72 h at 60°C. Dry samples were ground through a 2 mm screen using a Wiley Mill grinder, Philadelphia PA, and then ground through a 1 mm screen using an Arthur Thomas grinder, Philadelphia PA. Ground samples were stored in 120 ml airtight containers for subsequent analysis (Becton Dickinson). All samples were analyzed for acid detergent fiber (ADF) and neutral detergent fiber (NDF) using a modified method of Van Soest et al., (1991) called the filter bag technique (ANKOM Co., Fairport, NY) (Komarek et al., 1994). Samples were also analyzed for nitrogen using the Leco FP-428 Nitrogen analyzer (Leco Corp., St. Joseph, MI).

3.3.6 Blood Sampling and Analysis

Blood samples (10 ml) were taken by venipuncture from the jugular vein from each cow at seven and fourteen days post-partum in ethylenediamine tetra-acetic acid (EDTA) and heparinized 10 ml vacutainers (Becton Dickinson). Samples were taken between 10:30 A.M. and 11:30 A.M. on sampling days. Red blood cell percentage was determined by hematocrit analysis. Blood was then centrifuged for 10 min at 2500 rpm using a Beckman model J.6B centrifuge (Beckman instruments Inc., Palo Alto, CA). Plasma was then separated off and frozen in a fridge freezer at negative 14°C for further analysis. Blood urea nitrogen (BUN) and blood glucose (GLU) were determined using a Kodak Ektachem DT 60 Analyzer with Disc Two Module (Clinical Products Division, Eastman Kodak Co., Rochester, NY). Non-esterified fatty acid levels (NEFA) were determined using a NEFA kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan.).
3.3.7 Rumen Fluid Sampling

Rumen fluid was sampled at seven and fourteen days post-partum in conjunction with blood sampling. Sampling occurred two and a half hours post feeding, between 10:30 A.M. and 11:30 A.M. on sampling dates. Approximately 200 ml samples of rumen fluid were collected by vacuum stomach tube from each cow. Rumen fluid stored in 120 ml air tight plastic containers (Becton Dikinson) which were filled to the top, sealed and immediately placed on ice and transported to the laboratory for analysis. Rumen fluid pH was determined using a Corning electrode pH meter 130 (Medfield, MA, USA). Fluid was then centrifuged for 20 min at 3500 rpm using a refrigerated Beckman model J.6B centrifuge with a J.S. 4.2 rotor (Beckman instruments Inc., Palo Alto, California, USA). Four milliliters of the supernatant was removed and acidified by addition of 1 ml of 25% phosphoric acid. Samples were then frozen for further analysis. Volatile fatty acid (VFA) determination was conducted by using a Shimadzu gas chromatograph equipped with a capillary column (30 m x 0.25 mm I.D.Stabilwax-DA). Further detail can be found in Section 2.3.12.

3.3.8 Milk Sampling

Milk yield was recorded twice daily for fourteen days post partum and for a subsequent 60 days after the cows entered the main herd. Milk samples were taken for period one for four consecutive milkings on days thirteen and fourteen of period one. Milk samples were analysed for percentage of fat, protein, lactose, total solids and somatic cell count (SCC). For period two samples were taken from each cow for two consecutive milkings during herd milk testing DHIS dates and analysed for fat and protein percentages and somatic cell counts. Analysis of milk samples was conducted by Infra Red Milk Analysis System (B.C. DHIS Laboratory, Chilliwack, B.C.). Total daily percentages of milk composition and milk component yields were calculated using the A.M. and P.M. milk yield for the sampling date.
3.3.9 Efficiency

Milk yield and DMI for period one were used to calculate efficiency of cows using the equation:

\[ \text{Efficiency} = \frac{\text{milk weight (kg/d)}}{\text{dry matter intake (kg/d)}} \] (equation 4.0)

3.3.10 Body Condition Score

Body condition score (BCS) was used to evaluate body condition of the transition dairy cow. The BCS system used in the trial was based on a five point scale refined by increments of 0.25 to 0.5 units. Scoring was determined by visual and/or tactile appraisal of the amount of fat stored by the cow particularly over the bony prominence of the back and pelvic regions, hook bone, pin bone, tail head and rump (Ferguson et al., 1994, Gallo et al., 1996). Scoring was completed by the same observer when each cow started period one and again at seven and fourteen days post partum.

3.3.11 Statistical Analysis

Statistical analysis was conducted via least squares general linear model (GLM) procedures of SAS (1990). The following model was used for the analysis of pre partum dry matter intake, post partum intake, body weight, body condition score, blood urea nitrogen, glucose, hematocrit percentage, rumen pH, volatile fatty acid, milk composition and component yield:

\[ Y_{ijkl} = \mu + T_i + P_j + C_{k(l)} + e_{ijkl} \]

\( \mu \) = overall mean

\( T_i \) = effect of treatment, \( i = 1, \ldots 2 \)

\( P_k \) = effect of pair, \( k = 1, 2 \)

\( C_{i(l)} \) = effect of cow (\( l = 1, \ldots 12 \)) within pair \( k = 1, 2 \)

\( e_{ijkl} \) = experimental error
The model used for milk weight analysis for period one and period two was that of a split plot in time:

\[ Y_{ijklm} = u + T_i + P_j + C_{kl} + W_m + e_{ijklm} \]

\( u \) = overall mean
\( T_i \) = effect of treatment, \( i = 1,...,2 \)
\( P_j \) = effect of pair, \( k = 1,2 \)
\( C_{kl} \) = effect of cow (\( l = 1,...,12 \)) within pair \( k = 1,2 \)
\( W_m \) = effect of week, \( m = 1,...,12 \)
\( e_{ijklm} \) = experimental error

3.4 RESULTS

3.4.1 Feed Composition

Feed composition data are given in Tables 3.1 – 3.4. The basal ration for the pre partum period and period one was composed of 85% forage and 15 % barley based concentrate. Of the 85% forage, 47% consisted of corn silage and 38% consisted of grass silage on a dry matter basis. Dry matter content of the basal ration ingredients was 29.22 % for corn silage, 22.62 % for grass silage and 88% for concentrate (Table 3.1). Crude protein levels were 7.01 %, 10.25 % and 17.27 % for corn silage, grass silage and concentrate respectively (Table 3.1). Acid detergent fiber was determined to be 29.77 % for corn silage, 39.65 % for grass silage and 13.01 % for concentrate (Table 3.1). The neutral detergent fiber levels were 52.38 %, 58.59 % and 35.63 % for corn silage, grass silage and concentrate respectively (Table 3.1). Basal ration composition can be seen in Table 3.1. The ingredients of the diet were changed April 18th, 1997 due to a lack of grass silage. The new basal ration was composed of 85 % corn silage, and 15 % barley based concentrate. The dry matter content of corn silage and concentrate were 28.65 % and 89.41 % respectively (Table 3.2). Crude protein content was 6.47 % for corn silage and
16.32 % for the concentrate (Table 3.2). The acid detergent fiber was 31.52 % and 12.35 % for corn silage and concentrate respectively (Table 3.2). Neutral detergent fiber was determined to be 54.44 % for corn silage and 35.22 % for concentrate (Table 3.2).

The lactational diet of period one consisted of the basal ration mentioned above and additional concentrate increasing by increments of 0.5 kg/d from 0 – 14 DIM. The additional barley based concentrate was the same as that mixed in the basal ration (Table 3.1 and Table 3.2).

**3.4.2 Dietary Intakes**

Dry matter intake (DMI) for the days pre partum were not used in the statistical analysis because of the large variation in DMI during this time frame and the fact that the length of the pre partum period varied from 1 – 21 days. The average number of days for both treatment groups was 7 days, despite the large variation in the number of days prior to calving. The average DMI for the control cows was 8.3 kg/d and 9.1 kg/d for the LYC supplemented cows (Table 3.3). This average DMI was calculated from data for the seven days prior to calving date. Cows entered the pre partum period one week prior to expected calving date. However, due to unpredictability of calving time, cows remained in this period anywhere from 1-21 days. The time prior to calving can then be considered the adaptation period to LYC supplementation. Post calving the DMI was recorded daily for 14 days. There was no significant (P>0.05) difference in DMI among cows in control group compared to the LYC supplemented group (Table 3.5). However, a trend existed in the second week of period one in which LYC supplemented cows had higher DMI (Figure 3.1). Neither body weight change nor change in body condition score were significantly (P>0.05) affected by LYC supplementation (Table 3.5).
3.4.3 Blood Composition

Selected blood parameters of hematocrit, blood urea nitrogen, blood glucose and non-esterified fatty acids concentrations were not significantly (P>0.05) affected by the supplementation of LYC (Table 3.6).

3.4.4 Rumen Fluid pH

Rumen Fluid pH was unaffected by the supplementation of LYC (Table 3.7).

3.4.5 Volatile Fatty Acids

Rumen fluid samples for volatile fatty acid (VFA) analysis were taken on day seven and day fourteen of period one. Supplementation of LYC had no significant (P>0.05) effect on rumen acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, caproate, branched or total volatile fatty acids (VFA) or the acetate: propionate ratio (Table 3.7). Results indicate that there was no significant (P>0.05) difference between the LYC supplemented cows and the control cows for any molar percentage of VFA (Table 3.7). The total VFA production (mols) was not significantly (P>0.05) affected by LYC inclusion (Table 3.7). Analysis of VFA production for week one of period one indicated that there was no significant (P>0.05) difference between the total VFA or individual VFA production for the control or LYC supplemented cows (Table 3.8). For week two of period one there was no significant (P>0.05) effect of LYC supplementation on total VFA production or acetate, propionate, isobutyrate, butyrate, isovalerate, caproate, branched or acetate:propionate ratio. There was a significant (P<0.05) decrease (1.58 vs. 1.28 mol/100mol) in valeric acid when comparing the control to LYC supplemented cows, respectively (Table 3.9).
3.4.6 Milk Production Period One (0 – 14 DIM)

Total milk production was not significantly affected (P>0.05) by LYC supplementation (Table 3.10). Neither milk composition and or milk component yield of fat, protein, lactose and total solids were significantly influenced (P>0.05) by LYC supplementation (Table 3.10). Somatic cell count was also unaffected (P>0.05) by treatment (Table 3.7). Analysis of period one on a daily basis indicated that there was a significantly (P<0.05) increased (37.46 vs. 42.20 kg/d) milk yield for day 14 when LYC supplemented cows were compared to the control cows, respectively (Table 3.11, Figure 3.2).

3.4.7 Milk Production Period Two

Milk production for the 70 d period was significantly (P<0.05) higher for the previously supplemented LYC group compared to the control group (Table 3.12). The LYC supplemented group produced an average of 3.73 kg/d more milk yield than the control group. Milk composition and milk component yield for fat and protein were unaffected (P>0.05) by previous LYC supplementation (Table 3.12). Analysis of the period by week indicates that there was a significantly (P<0.05) higher milk yield for weeks 4, 6, 7, 8, 9, 10, 11 and 12 for the previously LYC supplemented cows compared to the control cows (Table 3.13, Figure 3.3). Analysis of the 305 d lactation indicated that the LYC supplemented cows had an earlier, higher and longer peak of lactation (Figure 3.4).

3.5 DISCUSSION

The ability of LYC to elicit a response in ruminants depends on a variety of factors such as the stage of lactation, type of yeast strain and most importantly the viability of yeast cells in the LYC product (Piva et al., 1989, Grings et al., 1992, Newbold 1995, and Kung et al., 1997). The yeast used in the current study was an active live yeast culture with a viable population of 1 x

Supplementation of LYC was at a level of 0.2% DM basis, based on estimated pre partum DMI, equaling an average of 33 g/d of LYC per cow. This inclusion amount is considerably higher than previous studies. On average, the literature has shown that inclusion of 10 g/d of LYC is adequate to elicit a response in lactating dairy cows (Gunther 1989, Huber et al., 1989, Wohlt et al., 1991, Erasmus et al., 1992, Kumar et al., 1992, Piva et al., 1993, Moloney and Drennan 1994, Chiquette 1995, and Wohlt et al., 1998). In the current study, an average of 18 g/d more of the LYC were incorporated into the transition dairy cow rations. This can be explained due to the activity of the yeast cells contained in the LYC supplement. In the current study the LYC was an active live yeast culture with a determined viable population of $1 \times 10^9$ cfu/g of yeast culture which would mean that if cows averaged 33 g/d of the LYC, technically the total viable population of yeast reaching the rumen should have $2.8 \times 10^{10}$ cfu. Previous studies have utilized 10 g/d of a higher activity LYC product ranging from $7.15 \times 10^8$ - $10 \times 10^9$ cfu/g creating conditions in the rumen in the range of $7.15 \times 10^9$ to $1 \times 10^{11}$ cfu (Wohlt et al., 1991, Kumar et al., 1992, Piva et al., 1993, Moloney and Drennan 1994, and Chiquette 1995). Therefore the amount of yeast actually entering into the rumen in the current study falls within the range of viable populations reported in previous studies.

Overall the inclusion of LYC one week prior to the expected calving date until 14 DIM did not elicit a significant ($P>0.05$) improvement in milk yield. However, in period one, as the period progressed it became evident that there was a trend towards higher milk yield. The inclusion of LYC on average produced a higher milk yield of 1.44 kg/d for the LYC supplemented cows compared to the control in period one. Although this increase in milk yield
was not significantly (P>0.05) higher it was greater than earlier responses seen with LYC in transition cow rations. Wohlt et al., 1998 and Robinson 1997 began inclusion of LYC in to the rations of transition cows 21 to 30 days prior to calving and both resulted in an increased milk yield ranging from 0.8 to 1.2 kg/d. By day 14 of period one the increase in milk yield was 3.69 kg/d, which was significantly (P<0.05) higher than the control group. This response is much greater than those seen with the majority of previous studies which have indicated that the inclusion of LYC had a positive influence on milk production ranging from 0.3 to 1.4 kg/d (Wohlt et al., 1991, Erasmus et al., 1992, Piva et al., 1993, and Putnam et al., 1997). LYC had no significant (P>0.05) effect on milk composition or milk component yield. This result is similar to studies by Erdman and Sharma 1989, Arambel and Kent 1990 and Chiquette 1995. These studies also showed no increase in milk yield.

Studies that have looked at the DMI prior to parturition Wohlt et al., (1991), Wohlt et al., (1998) and Robinson (1997), have shown that the inclusion of LYC in the pre partum period will not eliminate the drop in DMI but will reduce the magnitude of the decrease. Robinson (1997) and Wohlt et al., (1998) showed that with the inclusion of LYC there was no difference in DMI between the control or supplemented groups. Wohlt et al., (1991) showed that the LYC supplemented cows had slightly higher DMI of 0.4 kg/d. In the current study the LYC supplemented cows had a pre partum DMI that was 0.8 kg/d greater than the control. Although this increase in DMI was not significant it indicates that LYC has the potential ability to stimulate DMI pre partum (Table 3.3).

Previous studies that have claimed improved milk yield in dairy cows with supplementation of LYC have also shown an increased DMI (Wohlt et al., 1991 and Erasmus et al., 1992). In the current study the supplementation of LYC in period one had no significant (P>0.05) effect on post partum DMI (Table 3.5 or Figure 3.1). According to the literature the lack of increased milk production may be a direct reflection of a lack of an increase in feed intake. Similar results
have been found by studies conducted by Erdman and Sharma (1989), Piva et al., (1993) and Wohlt et al., (1998). An increased DMI ranging from 0.5 to 1.5 kg/d was found by Weidmeier et al., 1987, Williams and Newbold 1990, Williams et al., 1991, Wohlt et al., 1991, and Erasmus et al., 1992. It has been proposed that an increase in feed intake is due to the enhancement of ruminal conditions. The ability of LYC to improve the digestion in the rumen has been claimed by Arambel et al., 1987, Fallon and Harte 1987, Weidmeier et al., 1987, Williams et al., 1991, and Harris et al., 1992. Those studies claimed that digestion is better because of the stimulation of microbial growth.

LYC has the ability to influence conditions in the rumen which stimulates the total numbers of anaerobic bacteria (Weidmeier et al., 1987, Harrison et al., 1988, Edwards 1991, Wohlt et al., 1991, El Hassen et al., 1992, and Newbold et al., 1992). The exact mechanisms by which LYC enhances microbial growth is not known. A rise in the microbial population leads to a shift in fermentation end products (Dawson 1988). Microbes whose numbers are influenced by LYC supplementation are the cellulose and the lactic acid utilizing bacteria. Increased numbers of cellulolytic bacteria cause an increase in the rate of fiber digestion in forage but not the extent of degradation (Weidmeier et al., 1987, Arambel and Kent 1990, Dawson 1990, Wohlt et al., 1991, and Wallace and Newbold 1992). The increased rate of digestion leads to an increased rate of passage of feed and a subsequent increase in DMI (Gomez-Alarcon at al 1988). The rise in DMI leads to an increase in available nutrients to the animal and higher milk yields. The absence of increased DMI could be a direct reflection of the lack of significantly (P>0.05) improved efficiency for both control and supplemented cows. As mentioned earlier, LYC acts in the rumen to increase the rate of fiber digestion through increased numbers of microbes. Increased digestion would influence the efficiency of conversion of fiber into milk. Gunther, (1989), believes that LYC improves feed conversion for milk yield by increasing feed value. In the current study the lack of increased feed intake and efficiency may have been due to the lack
of increased anaerobic bacteria in the rumen. This is only a speculation since ruminal bacteria were not enumerated.

The inclusion of LYC had no effect on the blood non esterified fatty acids when compared to the control cows. Both supplemented cows and control cows were receiving adequate nutrients. This is also reflected in the lack of change in the body weight or body condition score. This absence of change in body weight and body condition score was seen in the previous studies of Erdman and Sharma (1989), Wohlt et al., (1991), Piva et al., (1993), Chiquette (1995) and Wohlt et al., (1998). The current study shows that the LYC supplementation had no adverse effect on the physical condition of the dairy cow.

Few studies have examined the effect that LYC could have on blood parameters of dairy cow. Of studies conducted which utilized LYC only three could be found that reported blood parameters Piva et al., 1993, Wohlt et al., 1991, and Wohlt et al., 1998. Their studies indicated that LYC inclusion had no influence on selected blood parameters such as blood urea nitrogen, blood glucose and hematocrit. These results are similar to results found in the current study in which no significant (P>0.05) differences were noted. This suggests that LYC supplementation has no effect on selected blood parameters.

The concentrations and proportions of volatile fatty acids (VFA) have been used to identify microbial activity in the rumen (Williams 1989). Studies in this area are inconsistent in results pertaining to the influence of LYC on VFA production. Numerous studies, both in vitro and in vivo, have been conducted utilising LYC and have reached the conclusion that LYC does not alter the total production of the VFA but does decrease the acetate to propionate ratio (Adams et al., 1981, Dawson and Newman 1987, Gray and Ryan 1987, Weidmeier et al., 1987, Dawson 1988, Martin et al., 1989, Williams 1989, Newbold et al., 1990, and Moloney and Drennan 1994). Other studies have shown that inclusion of LYC will increase the production of acetate (Weidmeier et al., 1987, and Piva et al., 1989). This contradiction confirms that the
effect of LYC on VFA responses is unclear. Of the studies mentioned above only a few (Piva et al., 1989, Piva et al., 1993, Chiquette 1995 and Putnam et al., 1997) were comparable to the current study in terms diet and DIM of the animals used. These four studies have found that the inclusion of LYC had no effect on the total or individual VFA production. Similarly, the current study found no significant (P>0.05) difference in total VFA, individual VFA production or acetate to propionate ratio.

Analysis of week one and week two of period one also showed no significant (P>0.05) alterations in the VFA production levels. An exception would be that of valerate, in week two of period one in which the LYC supplemented cows had significantly (P>0.05) lower levels of valerate than the control. No studies were found that documented valerate production levels. An increase in valerate production may or may not be a characteristic of LYC supplementation.

As mentioned above some studies have shown that with LYC supplementation there tends to be an rise in the concentration of acetate in the rumen fluid, resulting in an increased acetate to propionate ratio (Weidmeier et al., 1987 and Piva et al., 1989). The rise in acetate concentration is related to the increased in number of cellulolytic bacteria in the rumen (Weidmeier et al., 1987 and Williams 1989). As the numbers of cellulolytic bacteria increase more fiber is digested, thus producing more acetate (Thomas and Rook 1981). These studies did not show an increase in the total VFA, but the ratio of acetate to propionate had increased. There was a shift in the amounts of each VFA produced rather than accumulation of amounts of one particular VFA (Williams and Newbold 1990). The current study may not have shown change in VFA production due to the low concentrate diet. As the concentrate in the diet increases the LYC would influence numbers of cellulolytic bacteria, which would result in an increase in fiber digestion.

When considering the 14 days of period one individually, a distinct trend towards higher milk yield can be seen with LYC supplementation when compared to the control. LYC
supplemented cows reached significantly (P<0.05) higher milk production on day 14 of period one, compared to the control. Reasons why significance (P<0.05) was reached at the end of the LYC supplementation period are unclear. As the period progressed the level of concentrate increased at a rate of 0.5 kg/d for all cows. Cows started period one receiving barley based concentrate as a small portion of their ration. By day 7 the concentrate level had almost reached the level characteristic of the dietary intake of the main herd at 50% concentrate. Thus there was a gradual shift from low to high concentrate rations. There was an increase in concentrate fed from parturition to 14 DIM, of 5 kg/d to 11.5 kg/d. This rate of increase in concentrate intake simulates standard practices to decrease the chance of metabolic disorders such as acidosis when cows entered the main herd at 15 DIM and received a diet high in concentrate. Previous studies have indicated a better response to LYC inclusion with rations consisting of higher concentrate levels (Williams and Newbold 1990, Carro et al., 1992, and Piva et al., 1993). Studies claim that the response seen to LYC supplementation of lactating dairy cow rations is dependent on the basal diet (Moloney and Drennan 1994). When the concentrate to forage ratios of the rations are in the range of 50:50 to 60:40, the inclusion of LYC results in large increases in DMI and milk yield (Williams 1988).

Diets typically high in concentrate, particularly high starch diets of barley based concentrate, result in low ruminal pH, decreased acetate levels, increased propionate levels, decreases in cellulolysis and greater chances of acidosis (Thomas and Rook 1981, Chademana and Offer 1990, Moloney and Drennan 1994). Inclusion of LYC in these rations may help to alleviate some of these effects. Chiquette (1995) suggested that LYC aids in the metabolism of high concentrate diets by stabilization of the rumen pH. High lactic acid levels result from diets that are high in concentrate levels, which results in low ruminal pH. The lower pH caused by the accumulation of lactic acid can induce digestive disorders in the ruminant (Chaucheyras et al., 1995). The inclusion of LYC can decrease the levels of lactic acid and eliminate risks of
acidosis when diets high in readily fermentable carbohydrates are fed (Chaucheyras et al., 1995). Increases in the numbers of lactic acid utilizing bacteria decreases the level of lactic acid in the rumen and thus raises the pH (Harrison et al., 1988). Stabilization of ruminal pH provides an environment that is favourable for cellulolytic bacteria. A low ruminal pH inhibits cellulolytic bacteria and impairs fiber digestion (Dawson 1988). As the pH increases, cellulolytic bacteria become more active, enhancing fiber digestion. In the current study there was no significant (P>0.05) change in pH values. This was consistent with other studies of Piva et al., (1993) and Putnam et al., (1997). In those studies corn silage and barley concentrate based diets were similar to the diet of the current study. Harrison et al., (1988) and Williams and Newbold (1990) have shown an increase in pH with inclusion of LYC. As the concentrate in the diet is increased the pH drops and the benefits of LYC inclusion on stabilization of pH would be greater.

Researchers tend to agree that there is an interaction between LYC and concentrate content of the basal diet (Newbold et al., 1990, Williams and Newbold 1990, and Chiquette 1995). These claims help to explain why the early lactation cow showed no signs of improvement in milk yield, while consuming a diet low in concentrate. The LYC supplement may have been promoting more favorable conditions for the high concentrate rations near the end of the 14 d post partum. LYC primed the rumen of the supplemented cows for the high concentrate diets of the main herd, which would result in higher milk production.

LYC supplementation ceased after 14 DIM but milk yield and composition were measured for a subsequent 70 day period. During period two it was found that the cows previously supplemented with LYC had significantly (P<0.05) higher milk yield by an average of 3.7 kg/d compared to the control group. This increase was unexpected because previous work by Kung et al., (1997) indicated that after stopping LYC supplementation the population percentage of yeast cells in the rumen fluid decreased immediately and they were non-detectable by 24 h.
This indicated that LYC is essentially washed out of the rumen quickly, within one day. Any benefits that are directly related to the presence of LYC in the rumen should be eliminated. In the current study the most dramatic benefits were not seen during the supplementation, period one, but after supplementation stopped, period two. There was a trend towards higher milk yield for the previously supplemented LYC cows from week 3 through week 12 of lactation. This increase in milk yield reached significantly (P<0.05) higher levels at week 4 and from week 6 through week 12.

Cows previously supplemented with LYC peaked earlier and maintained peak lactation longer than the control group. This benefit of higher and longer peak lactation had been previously recorded by Wohlt et al., (1991). Higher and longer peaks enabled previously supplemented cows to maintain an advantage in milk production over the control cows. Wohlt et al., 1991, supplemented LYC through week 18 of lactation, while in the current study it was only included until 14 DIM. Wohlt et al., (1991) reported had an increase in milk yield that could be explained due to an increased DMI. In the current study, on day 15 of lactation cows on LYC supplementation entered the main herd and DMI was no longer monitored. Therefore it is unkown if an increased DMI, after 15 DIM, was the cause of the higher and longer peak in milk yield.

3.6 ECONOMIC BENEFITS

Through calculations using the Won at a rate of 7000W to 1.50 Canadian dollars an estimation can be made which reflects the cost and dollar benefits of LYC supplementation. CYC product is distributed at 3500W per ton of supplement. Conversions of this value to grams and Canadian funds values CYC at $ 0.008 per gram. The current study fed an average of 33 g per day of LYC for a total of 21 d. This resulted in a cost of $ 5.25 per cow. Financial benefit to the farmer can be based on an average increased milk production per cow of 1.4 kg/d from 0 – 14
DIM at a milk dollar value of $0.50/kg. Thus it can determined be determined that the farmer would receive a profit $4.55, minus labour, per cow fed LYC.

The current study found that after LYC supplementation ceased the benefit of increased milk yield continued with an average increased milk yield of 3.4 kg/d per cow. Considering the 21 d supplementation period and the 60 d non supplemental period for cows previously fed LYC. The profit per cow for the 60 d period would be $102.00, minus labour costs. Considering the two periods together the profit per cow would be $106.55, minus labour costs.

3.7 CONCLUSIONS

Inclusion of LYC supplementation prior to parturition was not significantly (P>0.05) beneficial for the first two weeks of early lactation, although there was a trend towards higher milk yield for the LYC supplemented cows. The benefits of LYC supplementation were observed by day fourteen. There was a dramatic increase in milk yield of 3.7 kg/d for week 3 through week 18 of lactation. There did not appear to be any adverse effects of this increased milk yield on the health of the LYC supplemented cows. It would therefore be beneficial to supplement cows during the transition from pregnancy to early lactation with 0.2% DM LYC beginning prior to parturition and ending during early lactation in order to elicit greater milk production. Future work should focus on the length of time LYC should be supplemented after parturition in order to elicit enhanced milk production.
3.7 REFERENCES


Grings, E.E, R.E Roffler, and D.P. Deitelhoff. 1992. Responses of dairy cows to addition of


Table 3.1  Nutrient composition (DM basis) of feeds from February 14 until April 18, 1997

<table>
<thead>
<tr>
<th>Nutrient$^{1,2}$</th>
<th>corn silage</th>
<th>grass silage</th>
<th>concentrate</th>
<th>hay</th>
<th>basal ration$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>% DM</td>
<td>29.22</td>
<td>22.62</td>
<td>88.03</td>
<td>88.42</td>
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<tr>
<td>% CP</td>
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<tr>
<td>% ADF</td>
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<td>29.97</td>
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<tr>
<td>% NDF</td>
<td>52.38</td>
<td>58.59</td>
<td>35.63</td>
<td>58.25</td>
<td>47.35</td>
</tr>
</tbody>
</table>

$^1$ DM, CP, ADF and NDF stand for dry matter, crude protein, acid detergent fiber and neutral detergent fiber respectively.

$^2$ total of 9 samples were taken

$^3$ total of 27 samples were taken
<table>
<thead>
<tr>
<th>Nutrient$^{1,2}$</th>
<th>corn silage</th>
<th>concentrate</th>
<th>hay</th>
<th>basal ration$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>% DM</td>
<td>28.65</td>
<td>89.41</td>
<td>83.10</td>
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<tr>
<td>% CP</td>
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<td>15.88</td>
<td>15.88</td>
</tr>
<tr>
<td>% ADF</td>
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<td>12.35</td>
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</tr>
<tr>
<td>% NDF</td>
<td>54.44</td>
<td>35.22</td>
<td>58.78</td>
<td>58.78</td>
</tr>
</tbody>
</table>

$^1$ DM, CP, ADF and NDF stand for dry matter, crude protein, acid detergent fiber and neutral detergent fiber respectively.

$^2$ total of 12 samples were taken

$^3$ total of 36 samples were taken
Table 3.3  Effect of LYC on pre partum dry matter intake.

<table>
<thead>
<tr>
<th>Cows</th>
<th>average treatment*</th>
<th>average days pre partum</th>
<th>dry matter intake (kg/d)</th>
<th>treatment dry matter intake (kg)</th>
<th>average pre partum body weight (kg)</th>
<th>intake expressed as % of body weight (intake/body weight)*100</th>
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<tbody>
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<td>n = 24</td>
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<td></td>
<td>8752</td>
<td>1</td>
<td>5</td>
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<td>816</td>
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</tbody>
</table>

* treatment 1 = control, treatment 2 = LYC supplementation
Table 3.4 Calculated amounts of LYC for LYC supplemented cows.

<table>
<thead>
<tr>
<th>Cows</th>
<th>Treatment*</th>
<th>pre partum average body weight (kg)</th>
<th>estimated DMI (kg/d)</th>
<th>LYC (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8733</td>
<td>2</td>
<td>812</td>
<td>16.24</td>
<td>36.0</td>
</tr>
<tr>
<td>8914</td>
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<td>826</td>
<td>16.52</td>
<td>36.7</td>
</tr>
<tr>
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<td>2</td>
<td>818</td>
<td>16.16</td>
<td>35.9</td>
</tr>
<tr>
<td>9204</td>
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<td>767</td>
<td>15.34</td>
<td>34.0</td>
</tr>
<tr>
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<td>763</td>
<td>15.26</td>
<td>33.9</td>
</tr>
<tr>
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<td>2</td>
<td>782</td>
<td>15.64</td>
<td>34.7</td>
</tr>
<tr>
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<td>692</td>
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<td>30.7</td>
</tr>
<tr>
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<td>723</td>
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<td>32.1</td>
</tr>
<tr>
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<td>709</td>
<td>14.18</td>
<td>31.5</td>
</tr>
<tr>
<td>92018</td>
<td>2</td>
<td>699</td>
<td>13.98</td>
<td>31.0</td>
</tr>
<tr>
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<td>699</td>
<td>13.98</td>
<td>31.0</td>
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<tr>
<td>94005</td>
<td>2</td>
<td>715</td>
<td>14.30</td>
<td>31.7</td>
</tr>
</tbody>
</table>

* treatment is LYC supplemented
Table 3.5  Influence of yeast supplementation on dry matter intake, body condition score and body weight for period one.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Yeast Supplementation</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI post-partum (kg/d)</td>
<td>17.72</td>
<td>17.40</td>
<td>+/- 0.36</td>
<td>0.5282</td>
</tr>
<tr>
<td>estimated DMI (kg, 2% BW)</td>
<td>14.55</td>
<td>14.87</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>LYC (g/d)</td>
<td>n/a</td>
<td>33.0</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>initial BW (kg)</td>
<td>727.50</td>
<td>743.50</td>
<td>+/- 19.5</td>
<td>0.5672</td>
</tr>
<tr>
<td>post BW (kg)</td>
<td>681.60</td>
<td>705.30</td>
<td>+/- 19.0</td>
<td>0.3886</td>
</tr>
<tr>
<td>change in BW (kg)</td>
<td>-45.92</td>
<td>-38.30</td>
<td>+/- 10.0</td>
<td>0.5962</td>
</tr>
<tr>
<td>initial BCS</td>
<td>2.96</td>
<td>3.16</td>
<td>+/- 0.08</td>
<td>0.1069</td>
</tr>
<tr>
<td>one week BCS</td>
<td>2.90</td>
<td>2.93</td>
<td>+/- 0.08</td>
<td>0.7841</td>
</tr>
<tr>
<td>two week BCS</td>
<td>2.90</td>
<td>2.91</td>
<td>+/- 0.08</td>
<td>0.9394</td>
</tr>
<tr>
<td>change in BCS</td>
<td>-0.06</td>
<td>-0.25</td>
<td>+/- 0.09</td>
<td>0.1693</td>
</tr>
<tr>
<td>efficiency (MW/DMI)</td>
<td>1.98</td>
<td>2.07</td>
<td>+/- 0.06</td>
<td>0.2656</td>
</tr>
</tbody>
</table>

a,b Means in the same row with different letters differ significantly (P<0.05).
Table 3.6 Effect of LYC supplementation on selected blood parameters for period one.

<table>
<thead>
<tr>
<th>Selected Blood Parameters</th>
<th>Control</th>
<th>Yeast Supplementation</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hematocrit (%)</td>
<td>32.04</td>
<td>32.35</td>
<td>+/- 0.53</td>
<td>0.6777</td>
</tr>
<tr>
<td>glucose (mg/dl)</td>
<td>59.83</td>
<td>63.29</td>
<td>+/- 2.71</td>
<td>0.3724</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>11.41</td>
<td>10.79</td>
<td>+/- 0.75</td>
<td>0.5595</td>
</tr>
<tr>
<td>NEFA (mEq/l)</td>
<td>0.93</td>
<td>1.11</td>
<td>+/- 0.11</td>
<td>0.3250</td>
</tr>
</tbody>
</table>

a,b Means in the same row with different letters differ significantly (P<0.05).
Table 3.7  Effect of LYC supplementation on rumen fluid pH and average volatile fatty acid concentration for period one.

<table>
<thead>
<tr>
<th>Volatile fatty acids</th>
<th>Control</th>
<th>Yeast Supplementation</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.86</td>
<td>6.96</td>
<td>+/- 0.08</td>
<td>0.3811</td>
</tr>
<tr>
<td>total VFA (mols)</td>
<td>63.46</td>
<td>60.84</td>
<td>+/- 2.70</td>
<td>0.5633</td>
</tr>
<tr>
<td>acetate (mol/100mol)</td>
<td>58.22</td>
<td>59.16</td>
<td>+/- 0.80</td>
<td>0.4164</td>
</tr>
<tr>
<td>propionate (mol/100mol)</td>
<td>24.99</td>
<td>24.94</td>
<td>+/- 0.85</td>
<td>0.9684</td>
</tr>
<tr>
<td>isobutyrate (mol/100mol)</td>
<td>0.61</td>
<td>0.57</td>
<td>+/- 0.02</td>
<td>0.2625</td>
</tr>
<tr>
<td>butyrate (mol/100mol)</td>
<td>12.78</td>
<td>12.19</td>
<td>+/- 0.37</td>
<td>0.2650</td>
</tr>
<tr>
<td>isovalerate (mol/100mol)</td>
<td>1.41</td>
<td>1.30</td>
<td>+/- 0.06</td>
<td>0.2123</td>
</tr>
<tr>
<td>valerate (mol/100mol)</td>
<td>1.51</td>
<td>1.34</td>
<td>+/- 0.06</td>
<td>0.0476</td>
</tr>
<tr>
<td>caproate (mol/100mol)</td>
<td>0.48</td>
<td>0.51</td>
<td>+/- 0.06</td>
<td>0.7277</td>
</tr>
<tr>
<td>branched(^1) (mol/100mol)</td>
<td>2.02</td>
<td>1.87</td>
<td>+/- 0.07</td>
<td>0.1445</td>
</tr>
<tr>
<td>a: p ratio(^2)</td>
<td>2.56</td>
<td>2.56</td>
<td>+/- 0.13</td>
<td>0.9976</td>
</tr>
</tbody>
</table>

\(^a,b\) Means in the same row with different letters differ significantly (P<0.05).

\(^1\) branched consists of the sum of isobutyrate and isovalerate

\(^2\) acetate:propionate ratio
Table 3.8  Effect of LYC supplementation on volatile fatty acid concentration for week one of period one.

<table>
<thead>
<tr>
<th>Volatile fatty acid</th>
<th>Control</th>
<th>Yeast Supplementation</th>
<th>SE</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>acetate (mol/100mol)</td>
<td>58.58</td>
<td>58.88</td>
<td>+/- 1.08</td>
<td>0.8468</td>
</tr>
<tr>
<td>propionate (mol/100mol)</td>
<td>24.27</td>
<td>25.06</td>
<td>+/- 1.10</td>
<td>0.6330</td>
</tr>
<tr>
<td>isobutyrate (mol/100mol)</td>
<td>0.59</td>
<td>0.58</td>
<td>+/- 0.26</td>
<td>0.8064</td>
</tr>
<tr>
<td>butyrate (mol/100mol)</td>
<td>13.27</td>
<td>13.25</td>
<td>+/- 0.51</td>
<td>0.1630</td>
</tr>
<tr>
<td>isovalerate (mol/100mol)</td>
<td>1.44</td>
<td>1.32</td>
<td>+/- 0.09</td>
<td>0.3706</td>
</tr>
<tr>
<td>valerate (mol/100mol)</td>
<td>1.44</td>
<td>1.39</td>
<td>+/- 0.08</td>
<td>0.6355</td>
</tr>
<tr>
<td>caproate (mol/100mol)</td>
<td>0.39</td>
<td>0.55</td>
<td>+/- 0.06</td>
<td>0.0918</td>
</tr>
<tr>
<td>branched(^1) (mol/100mol)</td>
<td>2.02</td>
<td>1.89</td>
<td>+/- 0.10</td>
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<tr>
<td>a: p ratio(^2)</td>
<td>2.63</td>
<td>2.50</td>
<td>+/- 0.15</td>
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<tr>
<td>total VFA (mols)</td>
<td>60.78</td>
<td>64.94</td>
<td>+/- 3.25</td>
<td>0.3813</td>
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</table>

\(^1\) branched consists of the sum of isobutyrate and isovalerate  
\(^2\) acetate:propionate ratio
Table 3.9  Effect of LYC supplementation on volatile fatty acid concentration for week two of period one.

<table>
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<th>Yeast Supplementation</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetate (mol/100mol)</td>
<td>57.61</td>
<td>59.48</td>
<td>+/- 1.18</td>
<td>0.2632</td>
</tr>
<tr>
<td>propionate (mol/100mol)</td>
<td>25.72</td>
<td>24.79</td>
<td>+/- 1.23</td>
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</tr>
<tr>
<td>isobutyrate (mol/100mol)</td>
<td>0.62</td>
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<td>+/- 0.04</td>
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<td>butyrate (mol/100mol)</td>
<td>12.50</td>
<td>12.13</td>
<td>+/- 0.52</td>
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</tr>
<tr>
<td>isovalerate (mol/100mol)</td>
<td>1.40</td>
<td>1.27</td>
<td>+/- 0.08</td>
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</tr>
<tr>
<td>valerate (mol/100mol)</td>
<td>1.58b</td>
<td>1.28a</td>
<td>+/- 0.07</td>
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<tr>
<td>caproate (mol/100mol)</td>
<td>0.54</td>
<td>0.46</td>
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</tr>
<tr>
<td>branched(^1) (mol/100mol)</td>
<td>2.03</td>
<td>1.84</td>
<td>+/- 0.10</td>
<td>0.1959</td>
</tr>
<tr>
<td>a: p ratio(^2)</td>
<td>2.46</td>
<td>2.64</td>
<td>+/- 0.21</td>
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<td>total VFA (mols)</td>
<td>66.00</td>
<td>56.19</td>
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</table>

\(^a,b\) Means in the same row with different letters differ significantly (P<0.05).
\(^1\) branched consists of the sum of isobutyrate and isovalerate
\(^2\) acetate:propionate ratio
Table 3.10  Milk yield and composition for period one.

<table>
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<th>Control</th>
<th>Yeast Supplementation</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>milk yield (kg/d) 0 - 14 DIM</td>
<td>32.8</td>
<td>34.24</td>
<td>+/- 0.66</td>
<td>0.1284</td>
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<tr>
<td>fat (%)</td>
<td>3.80</td>
<td>4.07</td>
<td>+/- 0.21</td>
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</tr>
<tr>
<td>fat (kg/d)</td>
<td>1.39</td>
<td>1.59</td>
<td>+/- 0.07</td>
<td>0.3091</td>
</tr>
<tr>
<td>protein (%)</td>
<td>3.29</td>
<td>3.22</td>
<td>+/- 0.07</td>
<td>0.3598</td>
</tr>
<tr>
<td>protein (kg/d)</td>
<td>1.22</td>
<td>1.26</td>
<td>+/- 0.04</td>
<td>0.7845</td>
</tr>
<tr>
<td>lactose (%)</td>
<td>4.73</td>
<td>4.61</td>
<td>+/- 0.07</td>
<td>0.1584</td>
</tr>
<tr>
<td>lactose (kg/d)</td>
<td>1.77</td>
<td>1.81</td>
<td>+/- 0.07</td>
<td>0.8349</td>
</tr>
<tr>
<td>total solids (%)</td>
<td>12.67</td>
<td>13.01</td>
<td>+/- 0.59</td>
<td>0.7682</td>
</tr>
<tr>
<td>total solids (kg/d)</td>
<td>4.67</td>
<td>5.05</td>
<td>+/- 0.20</td>
<td>0.3147</td>
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<tr>
<td>somatic cell count (x 10^3)</td>
<td>338.9</td>
<td>171.0</td>
<td>+/- 166</td>
<td>0.9182</td>
</tr>
</tbody>
</table>

a,b Means in the same row with different letters differ significantly (P<0.05).
<table>
<thead>
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<th>Control</th>
<th>Yeast Supplementation</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.15</td>
<td>11.21</td>
<td>+/- 2.26</td>
<td>0.9849</td>
</tr>
<tr>
<td>2</td>
<td>26.16</td>
<td>23.42</td>
<td>+/- 1.96</td>
<td>0.3375</td>
</tr>
<tr>
<td>3</td>
<td>28.30</td>
<td>33.30</td>
<td>+/- 1.84</td>
<td>0.0698</td>
</tr>
<tr>
<td>4</td>
<td>31.90</td>
<td>33.22</td>
<td>+/- 1.45</td>
<td>0.5303</td>
</tr>
<tr>
<td>5</td>
<td>31.86</td>
<td>32.81</td>
<td>+/- 1.65</td>
<td>0.6890</td>
</tr>
<tr>
<td>6</td>
<td>33.17</td>
<td>34.32</td>
<td>+/- 1.33</td>
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</tr>
<tr>
<td>7</td>
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<td>34.40</td>
<td>+/- 1.54</td>
<td>0.8946</td>
</tr>
<tr>
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<td>35.58</td>
<td>36.77</td>
<td>+/- 1.31</td>
<td>0.5299</td>
</tr>
<tr>
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<td>36.06</td>
<td>+/- 1.30</td>
<td>0.6355</td>
</tr>
<tr>
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<td>37.18</td>
<td>36.90</td>
<td>+/- 1.38</td>
<td>0.8859</td>
</tr>
<tr>
<td>11</td>
<td>36.03</td>
<td>39.68</td>
<td>+/- 1.46</td>
<td>0.0898</td>
</tr>
<tr>
<td>12</td>
<td>37.53</td>
<td>38.80</td>
<td>+/- 1.80</td>
<td>0.4857</td>
</tr>
<tr>
<td>13</td>
<td>37.55</td>
<td>39.38</td>
<td>+/- 1.40</td>
<td>0.3675</td>
</tr>
<tr>
<td>14</td>
<td>37.46a</td>
<td>42.20b</td>
<td>+/- 1.51</td>
<td>0.0361</td>
</tr>
</tbody>
</table>

a,b Means in the same row with different letters differ significantly (P<0.05).
Table 3.12 Milk yield and composition for period two.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Yeast Supplementation</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>milk yield (kg/d) 15 – 85 DIM</td>
<td>40.36a</td>
<td>44.09b</td>
<td>+/- 0.32</td>
<td>0.0385</td>
</tr>
<tr>
<td>fat (%)</td>
<td>3.22</td>
<td>3.21</td>
<td>+/- 0.15</td>
<td>0.9342</td>
</tr>
<tr>
<td>fat (kg)</td>
<td>1.30</td>
<td>1.42</td>
<td>+/- 0.06</td>
<td>0.2159</td>
</tr>
<tr>
<td>protein (%)</td>
<td>2.93</td>
<td>3.00</td>
<td>+/- 0.04</td>
<td>0.2612</td>
</tr>
<tr>
<td>protein (kg)</td>
<td>1.18</td>
<td>1.32</td>
<td>+/- 0.04</td>
<td>0.0667</td>
</tr>
</tbody>
</table>

a,b Means in the same row with different letters differ significantly (P<0.05).
Table 3.13  Average weekly milk yield for period one and period two.

<table>
<thead>
<tr>
<th>Week of lactation</th>
<th>Control</th>
<th>Yeast Supplementation</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29.16a</td>
<td>29.16a</td>
<td>+/- 1.00</td>
<td>0.9986</td>
</tr>
<tr>
<td>2</td>
<td>36.45b</td>
<td>39.08bc</td>
<td>+/- 1.00</td>
<td>0.0662</td>
</tr>
<tr>
<td>3</td>
<td>40.01c</td>
<td>41.98cdef</td>
<td>+/- 1.00</td>
<td>0.1698</td>
</tr>
<tr>
<td>4</td>
<td>41.7cde</td>
<td>44.70fghi</td>
<td>+/- 1.00</td>
<td>0.0423</td>
</tr>
<tr>
<td>5</td>
<td>43.77defgh</td>
<td>45.64ghij</td>
<td>+/- 1.00</td>
<td>0.2033</td>
</tr>
<tr>
<td>6</td>
<td>43.80defgh</td>
<td>47.49ij</td>
<td>+/- 1.00</td>
<td>0.0126</td>
</tr>
<tr>
<td>7</td>
<td>43.92efgh</td>
<td>48.06j</td>
<td>+/- 1.00</td>
<td>0.0053</td>
</tr>
<tr>
<td>8</td>
<td>42.03cdef</td>
<td>48.00j</td>
<td>+/- 1.05</td>
<td>0.0001</td>
</tr>
<tr>
<td>9</td>
<td>40.79cd</td>
<td>48.00j</td>
<td>+/- 1.10</td>
<td>0.0001</td>
</tr>
<tr>
<td>10</td>
<td>41.42cde</td>
<td>46.99ij</td>
<td>+/- 1.10</td>
<td>0.0001</td>
</tr>
<tr>
<td>11</td>
<td>41.08cde</td>
<td>46.52hij</td>
<td>+/- 1.10</td>
<td>0.0003</td>
</tr>
<tr>
<td>12</td>
<td>40.13c</td>
<td>43.44defg</td>
<td>+/- 1.10</td>
<td>0.0394</td>
</tr>
</tbody>
</table>

a,b,c,d,e,f,g,h,i,j  LSM in the same column with different letters differ significantly (P<0.05).
Figure 3.1 Effect of yeast supplementation on daily dry matter intake for period one.
Figure 3.2 Effect of LYC supplementation on daily milk yield for period one
Figure 3.3 Effect of LYC supplementation on average milk weight for period one and period two.
Figure 3.4 Effect of live yeast culture on 305 d milk yield
4.1 FUTURE RESEARCH

The current study has shown that the inclusion of LYC in rations of early lactation dairy cows is not detrimental to the cow and is financially beneficial for the farmer. Further research is needed to determine the practical application of LYC supplementation on both small scale and large dairy farms. For LYC inclusion to become a common practice it needs to be easily fit into the current feeding strategies and management practices of the farm.

From the current study it was determined that LYC was beneficial to the early production of dairy cows beginning from 7 d pre partum. Future studies should investigate the time duration that LYC should be fed prior to parturition. Questions arose as to whether 7 d was adequate or should it be fed for longer 14 – 21 days pre partum. As well it would be beneficial to investigate if LYC needed to be included prior to parturition or if beginning supplementation at parturition would be adequate and continue for a range of 7 – 21 days. Differing levels of LYC could be compared. The level of LYC inclusion in the current study was determined through \textit{in vitro} techniques but it is possible that lower levels could elicit similar responses in early lactation dairy cows. There are several means by which this research could be expanded on in the future, only some have been briefly mentioned.

4.2 GENERAL CONCLUSIONS

The current study demonstrated that the use of an \textit{in vitro} fermentation technique, gas measurement machine, was a good procedure to assist in determining which level of LYC would potentially produce the most optimal fermentation conditions in the rumen of a dairy cow. From these experimentations the inclusion level of 0.2% LYC, on a DM basis, was determined to be the best level for inclusion in a dairy ration. Subsequent lactational experimentation demonstrated that LYC can result in dramatic increases in milk production from dairy cows.
during their transition from late pregnancy to early lactation. The addition of LYC into the
ration of these transition cows prior to parturition, at a level of 0.2% DM basis, increased the
milk yield by an average of 1.4 kg/d. After supplementation ceased, the benefit from LYC was
still observed with an average increase in yield by 3.4 kg/d. From these studies the exact mode
of action of the LYC in the rumen is still not known but these studies have contributed to a better
understanding of the conditions in which LYC elicits the best responses. Use of LYC dairy
rations, not only lactating but transition cows, provides opportunities for future practical
uses on dairy farms.
APPENDIX 1.0 Composition of reducing solution for *in vitro* gas production

**Main element solution**

5.7 g $\text{N}_2\text{HPO}_4$

6.2 g $\text{KH}_2\text{PO}_4$

0.6 g $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$

**Trace element solution**

13.2 g $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$

10.0 g $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$

1.0 g $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$

0.8 g $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$

make up to 100 ml with distilled water

**Buffer solution**

35 g $\text{NaHCO}_3$

4 g $(\text{NH}_4)\text{HCO}_3$

**Resazurin solution**

100 mg resazurin

make up to 100 ml with distilled water
Reducing solution

First 2 ml 1 M NaOH and then 285 mg Na₂S.7H₂O are added to 47.5 ml distilled water. This reducing solution must be freshly prepared each time shortly before the rumen fluid is removed. The other solutions can be made up and stored. The solutions are poured into a 4 L flask, mixed with a magnetic stirrer and heated to 39°C in a incubator. The solutions are added in the following order:

- 474 ml distilled water
- 0.12 ml trace element solution
- 237 ml buffer solution
- 237 ml main element solution
- 1.22 ml resazurin solution

Reference

Appendix 2.0 Regression analysis

<table>
<thead>
<tr>
<th>CO₂ injected (ml)</th>
<th>amount CO₂ dissolved in change</th>
<th>CO₂ available for pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 ml</td>
<td>1.14 ml</td>
<td>3.89 ml</td>
</tr>
<tr>
<td>10 ml</td>
<td>2.27 ml</td>
<td>7.78 ml</td>
</tr>
<tr>
<td>15 ml</td>
<td>3.40 ml</td>
<td>11.67 ml</td>
</tr>
<tr>
<td>20 ml</td>
<td>4.54 ml</td>
<td>15.56 ml</td>
</tr>
</tbody>
</table>

The voltage response from the gas available to induce change in pressure

<table>
<thead>
<tr>
<th>Volume of CO₂ (dependent Y)</th>
<th>Voltage response (independent X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.89 ml</td>
<td>0.6078 V</td>
</tr>
<tr>
<td>7.78 ml</td>
<td>0.6356 V</td>
</tr>
<tr>
<td>11.67 ml</td>
<td>0.6718 V</td>
</tr>
<tr>
<td>15.56 ml</td>
<td>0.7337 V</td>
</tr>
</tbody>
</table>

From the above information a regression equation can be developed so that the conversion from voltage to ml gas can be determined. The equation developed does not go through the origin.

\[ Y = mX + b \]

where

\[ X = \text{voltage response (V)} \]
\[ Y = \text{ml of CO₂} \]
\[ m = \text{slope of the line} \]
\[ b = \text{y-intercept} \]

and

\[ m = 72.77 \text{ ml} +/-.9.9 \]
\[ b = -43.13 \text{ ml} +/-.6.4 \]
Appendix 3.0 Economic benefits for production trial

Conversion to Canadian dollars:

$700W = $1.00 US = $1.50 CD

Cost CYC:

$3500W/kg = $7.50/kg CD
$7.50/kg CD = $0.0075/g CD
average 33g/d/cow = $0.25/d/cow
fed for 21 d * $0.25/d/cow = $5.25/cow

Returns for 21 d feeding for 12 cows:

0-14 DIM increased average 1.4kg/d milk
1.4kg/d milk * 14DIM = 19.6 kg milk
19.6 kg milk * $0.50 milk price = $9.80 0-14DIM
$9.80 0-14DIM - $5.25 cost CYC = $4.55 profit (minus labour)

Returns after CYC ceased after feeding 21d:

15-84 DIM increased milk average 3.4 kg/d * 60 d = 204 kg milk
204 kg milk * $0.50 milk price = $102.00 15-84DIM
$102.00 15-84DIM + $4.55 0-14DIM = $106.55 profit (minus labour) 0-84DIM