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Effects of Enzymatically Hydrolyzed Yeast Supplementation and Supplementation Frequency on Immune Parameters, Performance, and Digestibility among Periparturient Beef Cows and Calves

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EFFECTS OF ENZYMATICALLY HYDROLYZED YEAST SUPPLEMENTATION AND SUPPLEMENTATION FREQUENCY ON IMMUNE PARAMETERS, PERFORMANCE, AND DIGESTIBILITY AMONG PERIPARTURIENT BEEF COWS AND CALVES

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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TABLE OF CONTENTS

ABSTRACT

EFFECTS OF ENZYMATICALLY HYDROLYZED YEAST SUPPLEMENTATION AND SUPPLEMENTATION FREQUENCY ON IMMUNE PARAMETERS AMONG PERIPARTURIENT BEEF COWS AND CALVES

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Effects of enzymatically hydrolyzed yeast (EHY) and supplementation frequency (FREQ) on immune parameters among beef cows and calves was evaluated. Eighty multiparous (parity = 4.2 ± 0.3) cows were fed a common brome hay-based diet (CP = 8.0 ± 0.17%). Cows were blocked by expected calving date and stratified by body condition score (BCS) before random assignment of treatment. Beginning 88 ± 5 d prior to parturition, cows were provided 1 kg daily or 3 kg every 3 d of a soybean hull-based supplement (CP = 34.0, % of DM) that contained 0 or 3 g/kg EHY. The daily supplement was designed to meet ruminal N requirements (total or DIP?). Cows were vaccinated against rotavirus at 62 and 48 ± 5d prior to parturition. Sera and plasma samples were collected at 62, 48, 40, 24 and 14 d prior to parturition. At parturition, colostrum was collected via portable milking unit from cows prior to feeding to calves and plasma and sera was collected from cows and calves. Subsequently, calf plasma was collected at 2 and 14 d after parturition. Calf plasma Immunoglobulin G (IgG) concentration increased (*quadratic* < 0.01) as age increased and passive transfer status among calves was 'excellent' (i.e. plasma IgG concentration was greater than 15 g/L among calves aged 2 d;

APHIS, 2010). Plasma IgG was greater ($P = 0.03$) among calves born to cows receiving EHY and FREQ had no effect on plasma IgG in calves. Despite differences among calf plasma IgG concentrations, there was no effect of EHY or FREQ on colostrum yield, colostral IgG or calf intake of colostrum. Similarly, apparent efficiency of IgG absorption (AEA) and sera rotavirus neutralization titers (RNT) among calves aged 14 d was not affected by treatment ($P \geq 0.36$). Cow plasma IgG decreased (*quadratic* = 0.02) as cows neared parturition and were not affected by EHY ($P = 0.56$) or FREQ ($P = 0.14$). We observed a quadratic increase in rotavirus neutralization titers in cow sera in response to vaccination, as expected. Sera rotavirus neutralization titers were not impacted by EHY ($P = 0.70$) nor FREQ ($P = 0.42$).

Effects of enzymatically hydrolyzed yeast (EHY) and supplementation frequency on cow BW, cow BCS, calf BW, and cow milk production was evaluated. The same 80 multiparous (parity = 4.2 ± 0.3) cows were fed a common brome hay-based diet (CP = 8.0 ± 0.17%). Cows were blocked by expected calving date and stratified by BCS before random assignment of treatment. Beginning 88 ± 5 d prior to parturition, cows were provided 1 kg daily or 3 kg every 3 d of a soybean hull-based supplement (CP = 34% of DM) that contained 0 or 3 g/kg EHY. The daily supplement was designed to meet ruminal N requirements (total or DIP?). Cows were weighed on 88, 62, 48, 40, 24, 14 and 0 d prior to parturition. Although cow BW was recorded, it was not analyzed (Why?). Three trained technicians recorded BCS on the same day. After calving, milk production was recorded on 30, 90, and 150 d. Samples of milk from each cow were sent to Dairy Herd Improvement Agency (DHIA) for analysis. Cow BCS increased (*linear* < 0.01) as cows neared parturition. Frequency of supplementation tended ($P < 0.06$) to increase

BCS, whereas, effect of supplementation with EHY ($P < 0.91$) on cow BCS was not significant. Total cow milk yield (kg/d) decreased (*quadratic* < 0.01) by day. Neither FREQ ($P = 0.23$) nor EHY ($P = 0.59$) ~~significantly~~ affected total milk yield. Milk fat yield, milk protein yield, and energy corrected milk (ECM) yield was calculated. Milk fat yield decreased (*quadratic* < 0.01) by day. Neither FREQ ($P = 0.34$) nor EHY ($P = 0.63$) effected milk fat yield. Energy corrected milk yield decreased (*quadratic* < 0.01) by day and neither FREQ ($P = 0.25$) or EHY ($P = 0.78$) affected ECM. Milk protein yield was not affected by FREQ ($P = 0.13$) or EHY ($P = 0.69$) but was affected by d (*quadratic* < 0.01).

KEY WORDS: cattle, supplementation frequency, yeast

Literature Review

I. Passive immunity in cattle

Passive transfer of immunity is the transfer of maternal antibodies to naïve neonates. In cattle, passive transfer of immunity occurs in consumption and absorption of immunoglobulins (e.g., IgG) in colostrum. Passive transfer of immunity in cattle is essential to mitigating morbidity and mortality among neonatal calves. Calves must receive colostrum within the first 24 h of life for IgG immunoglobulins to be absorbed by the gut (Stott et al., 1979).

Immunoglobulins help to protect calves from pathogens early in life or until the calf's immune system matures and becomes protective, which can take weeks to months (Boyd, 1972; McGuire et al., 1976; Banks, 1982;). Passive transfer of immunity can have long-term impact among performance in cattle (Robison et al., 1988; Wittum and Perino, 1995; Faber, 2005). Failure of passive transfer of immunity increases calf morbidity and treatment cost (Faber, 2005; Priestley et al., 2013).

Failure of passive transfer among cattle contributes to a large number of preweaning deaths. Among the 34.3 million calves born in the U.S. (NASS, 2016) about 3.5% of calves born alive die before weaning (APHIS, 2010). About one-third (36.7%) of calves that die prior to 3 weeks of age (APHIS, 2010). Frequently, causes of death among calves that die prior to 3 weeks of age are digestive problems (e.g., diarrhea: 14.0%) and respiratory problems (e.g., pneumonia; 8.2%). If it is assumed that calves that die prior to 3 weeks of age as a result of digestive and respiratory problems ~~are~~ related to failure to passive transfer of immunity, then nearly 100,000 calves likely die in the United States annually from failed passive transfer of immunity.

Inclusion of yeast products in cattle diets may augment performance, digestion, or immunity. There are various types of yeast related products on the market in the United States: live yeast culture, killed yeast, hydrolyzed yeast cell walls, and even components of yeast cell walls. In regards to digestion, yeast in forms of active dry yeast and killed dry yeast, have been shown to increase ruminal pH. Higher ruminal pH optimizes the environment within the rumen allowing for better digestion and reduces acidotic conditions (Vyas et al., 2013). This may explain how yeast augments performance. However, yeast's mode of action on augmenting immunity in cattle has yet to be determined.

II. Effects of yeast on immunity

Transition from gestation to lactation can affect health status of cows. Subsequent deficiencies in energy and nutrients can decrease immune function (Goff and Horst, 1997). During transition from gestation to lactation, most dairy cattle are unable to consume adequate amounts of diet to meet energy requirements (NRC, 2001). Thus, cattle may benefit from augmented immune function during the periparturient period. Yuan et al. (2014) fed a yeast supplement to transition dairy cows to study effects on immune function. Bacteria exposed to whole blood from cows fed yeast had greater death rate with greater amount of yeast fed (Yuan et al., 2015). Also, yeast supplementation tended ($P < 0.01$) to increase plasma IgG. Similarly, Campus-Granados et al. (2013) reported that total immunoglobulins in colostrum increased among cattle fed partially hydrolyzed yeast. Further, there was less incidence of pneumonia or scours among calves born to cows fed partially hydrolyzed yeast (Campos-Granados et al., 2013).

Mannan oligosaccharides (MOS) are a component of yeast cell walls. *Saccharomyces* cerevisiae cells are lysed, the fragments of the cell wall are then centrifuged, washed and spray dried (Spring et al., 2000). Calves from cows fed MOS have a greater ability to neutralize foreign agents and this may contribute to decreased incidence of morbidity. Rotavirus neutralization titers measure ability of serum immune cells to neutralize a virus (Mak et al., 2014). Neutralization of a virus disables the virus's ability to infect cells and animals. Immunoglobulins have the ability to neutralize antigens such as viruses, along with neutrophils, monocytes, natural killer cells, macrophages, CD4+ T cells, CD8+ T cells, B cells, and dendritic cells (Mak et al., 2014). Franklin et al. (2005), studied effects of MOS on immunity in cows and calves. Cow rotavirus neutralization titers were greater ($P = 0.04$) among cows fed mannan oligosaccharides during the last 3 weeks of the dry period (Franklin et al., 2005). Calves from cows fed control, tended ($P = 0.05$) to have greater serum IgA than calves from cows fed MOS (Franklin et al., 2005). Immunoglobulin A is an immunoglobulin, like IgG, that the body produces in response to a foreign agent. However, immunoglobulin A is the last isotype to be formed after exposure to an antigen (Mak et al., 2014). Immunoglobulin A, in calves, is absorbed nonselectively into the blood (Tizard et al., 1996). Serum rotavirus neutralization titers tended ($P = 0.08$) to be greater in calves from cows fed MOS (Franklin et al., 2005).

Magalhães et al. (2008) fed yeast culture (YC) to dairy calves. Serum titers for anti-OVA IgG in calves were not affected ($P = 0.13$) by YC. Neutrophil function from calves at 25 d of age was not affected by YC. However, when neutrophils were exposed to E. coli, there was a tendency for the neutrophils from calves fed YC to have a greater

number of bacteria phagocytized. In addition, calves provided YC tended ($P = 0.06$) to have a smaller fecal score (1 = firm to 4 = watery and profuse diarrhea). These authors speculated that firmer feces are associated with healthier calves.

Heinrichs et al. (2003) fed milk replacer enhanced with MOS to dairy calves to compare effects of MOS, antibiotics (4 g of Bioneomycin + 200 g/ton oxytetracycline) and control. Heinrichs et al. (2003) measured fecal scores in calves. Calf fecal score can be an indicator of health or illness. Both antibiotics and MOS treatments were more likely ($P < 0.01$) to have normal feces or lower fecal scores. Compared to the control, both MOS and antibiotics decreased the likelihood of calves' feces being soft and runny. Of calves that scoured, those on either antibiotic treatment or MOS treatment, when compared to controls, recovered at a faster rate. Dry matter intake was increased ($P < 0.05$; 0.94 ± 0.05 kg/d) among calves fed MOS compared to antibiotics and control which were not different. Also, grain intake increased ($P < 0.05$) at a greater rate among calves provided MOS (Heinrich et al., 2003). Increasing rate of grain intake may improve nutrient balance in young calves and decrease morbidity and mortality.

Ponce et al. (2012) fed beef heifers (BW = 191 ± 1.17 kg) yeast and evaluated effects on performance and morbidity. Heifers fed yeast had a tendency ($P = 0.09$) to be less likely to be treated for bovine respiratory disease (BRD) (Ponce et al., 2012). This tendency to decrease morbidity by yeast may suggest improved immune response to vaccination. Additionally, heifers fed yeast had a tendency ($P = 0.07$) for greater ADG; however, there were no differences among DMI or feed efficiency (Ponce et al., 2012). It is possible that decreases in morbidity among heifers fed yeast were the result of an

increase in immune response. Subsequently, potential effects on immune function may contribute to differences in ADG.

Keyser et al. (2007) fed live yeast to heifers and studied effects on health and performance during the receiving period. Heifers received an oral paste of yeast (1 g) and fed 0.5 g/d yeast compared to heifers that received 1 mL water at processing and were not fed yeast. A lesser proportion ($P = 0.04$) of yeast fed cattle were treated for BRD than control (Keyser et al., 2007). Also, DMI tended to be greater among yeast fed heifers from d 0 to 28 ($P = 0.12$) and in d 0 to 35 ($P = 0.24$).

Malaczewska et al. (2010) studied effects of dried brewer's yeast fed to lambs aged 30 d. Phagocytic activity of lamb phagocytes was determined by the measurement of respiratory burst activity after stimulation with phorobol myristate acetate (Chung and Secombes, 1988; Siwicki et al., 1998). Phagocyte potential killing activity was measured by isolated blood leukocytes that were stimulated with killed microorganisms (Rook et al., 1995; Siwicki et al., 1998). Lamb phagocytes had greater metabolic activity ($P \leq 0.05$) and potential killing ability ($P \leq 0.01$) among lambs fed yeast. A similar increase ($P = 0.04$) was also found in lymphocyte proliferation (Malaczewska and Milewski, 2010).

Cole et al. (1992) studied effects of yeast culture on feeder calves and lambs. These authors (Cole et al., 1992) reported that dietary yeast culture did not affect morbidity, mortality, or performance of calves. However, in a different study, these authors (Cole et al., 1992) observed that fewer (5.72 versus 6.10) days ($P < 0.05$) on antibiotic therapy were needed to treat morbid calves compared to control (Cole et al., 1992). Additionally, calves fed yeast culture had greater DMI following an infectious bovine rhinotracheitis virus (IBRV) challenge; however, yeast did not affect calf rectal

temperature. Nonetheless, calves fed yeast culture tended ($P = 0.08$) to lose less weight in response to an IBRV challenge in comparison to controls. These results suggest that feeding yeast culture may provide benefit when calves are challenged with an infection agent.

Seymour et al. (1995) fed calves 1 of 4 pre-weaning treatments and 1 of 8 post-weaning treatments. Two of the 4 pre-weaning treatments contained brewer's yeast supplement, one being colostrum and the other being colostrum substitute. Calves were subsequently divided into 2 groups to accommodate 2 post-weaning treatments of yeast inclusion or no yeast inclusion within pre-weaning treatments. Calves were fed milk replacer twice a day according to manufacturer recommendation (0.227 kg of powder and 1.82 kg of liquid) from 5 d to 46 d of age twice daily and offered pelleted calf feed and water from d 5 to d 90 to ad libitum (Seymour et al., 1995). Calves were weighed weekly, scour score and respiratory condition was recorded twice daily, and body temperatures were recorded daily. Calves fed yeast had fewer days with an elevated body temperature between 12 and 25 d when compared to calves fed the control diet (Seymour et al., 1995). There were greater incidences of scours, milk refusals, and elevated temperatures between d 12 to d 25. However, calves fed yeast tended ($P = 0.07$) to have lower incidence of these measures. These calves experienced lower incidence of elevated body temperature, lower percentage of days with scours, and less treatment with antibiotics than calves fed treatments that did not include yeast. Later, between 26 and 46 d, these parameters decreased drastically. The control treatment (colostrum with no yeast) still had observed elevated temperatures and antibiotic treatments associated with treating elevated temperatures during period 4. Throughout the entire pre-weaning period, d 1 to d

46, inclusion of yeast in the calves' diet had a reduction ($P < 0.05$) on percentage of days with an elevated temperature and treatment with antibiotics ($P = 0.06$).

Galvão et al. (2005) evaluated effects of yeast on feces among calves with failed passive transfer of immunity. Live yeast was added to grain (SC), milk replacer (SB), both (SCSB) or none (control). Fecal scores were not affected by addition of live yeast to calf diets. Fecal scores were generally low in calves (Galvão et al., 2005). Prior to weaning, fewer days of diarrhea were observed in calves receiving diets containing SC and SB. After weaning, SC and SCSB fed calves were observed to have fewer days with diarrhea when compared to calves fed the control diet (Galvão et al., 2005). Despite fewer days of diarrhea in treatment calves, total cost of diarrhea associated treatment was not affected by treatment with live yeast. Fecal samples were cultured and tested for antibiotic susceptibility. According to Galvão et al. (2005) isolates from d 13 had high resistance. Antibiotic resistance was affected by SCSB treatment, increasing odds of multiple antibiotic resistance in fecal *E. coli*.

Nocek et al. (2011) fed lactating cows no yeast, yeast culture (YC), or yeast culture with enzymatically hydrolyzed yeast (YC+EHY). Milk somatic cell count decreased among cows fed YC+EHY when compared to the control and YC fed cows. New clinical cases of mastitis were found to be numerically less (5 vs. 10) in cows fed YC+EHY. Between weeks 8 and 14 postpartum, YC+EHY fed cows had decreased somatic cell count when compared control and YC fed cows.

Hill et al. (2009) supplemented calves with live yeast or mannan oligosaccharides. Live yeast (YST) and MOS did not affect respiratory scores ($P = 0.86$) or rectal temperatures ($P = 0.80$). However, fecal scores were affected ($P = 0.01$) by

supplementation. Holstein calves that received YST or MOS had lesser average fecal scores when compared to calves not receiving either supplement. In Jersey calves a similar affect was seen when the calves were fed YST. Jersey calves receiving MOS were not different from calves not receiving any supplement. However, also noted was the fact that all calves, no matter their assigned treatment, were generally healthy (Hill et al., 2009).

III. Yeast effects on performance

Williams et al. (1991) studied effects of yeast on dairy cow milk yield. Yeast was top dressed to a subsection of cows on each of the 4 diets (Williams et al., 1991). In diets high in concentrate, yeast had an increased effect ($P < 0.06$) on fat-corrected milk yield. Yeast culture did not affect milk yield in the treated straw diet. However, it did have a tendency to increase concentration of milk fat (Williams et al., 1991). Milk fat concentration tended to decrease when hay was the roughage in the diet. Yeast increased (27.4 ± 0.745 kg/d vs 23.3 kg/d, $P < 0.05$) milk yield among cows fed 60% concentrate and 40% hay diet (Williams et al., 1991). Cows receiving high-concentrate plus yeast culture had increased ($P < 0.05$) milk protein yields. Cows fed yeast tended to have greater live weight gains. Increased feed intake, which also may have resulted in greater milk yield (Williams et al., 1991).

Dann et al. (1999) reported that BCS was not affected ($P \geq 0.67$) by yeast before or after parturition. Yeast did not affect ($P > 0.10$) average daily milk yield. Interestingly, peak milk yield (*Yeast* × time = 0.01) occurred nearer to parturition (43 vs. 57 DIM) among cows fed yeast culture (Dann et al., 1999). However, milk composition was not affected ($P > 0.10$) by yeast, and yeast did not affect incidence of milk fever, ketosis,

retained placenta, displaced abomasum, mastitis, and postpartum anestrus interval (Dann et al., 1999).

Development and growth of neonatal calves is important to the cattle industry. Lesmeister et al. (2004) reported that yeast fed with milk replacer to suckling calves did not have an effect on the feed efficiency prior to weaning ($P > 0.05$). Total dry matter intake included dry matter from milk replacer and starter grain consumed. Greater yeast inclusion tended to increase ($P = 0.07$) starter DMI prior to weaning and increased ($P < 0.02$) starter DMI post-weaning. The lesser inclusion rate of yeast did not differ ($P > 0.05$) from control or the greater yeast inclusion. Average daily gain tended ($P = 0.06$) to be greater among calves fed greater amounts of yeast prior to weaning. Similarly, greater amounts of yeast inclusion increased ADG post-weaning (Lesmeister et al., 2004). Effects of yeast on ADG contributed to greater final BW among calves receiving greater amounts of yeast ($P < 0.03$). Also, feed efficiency, average daily hip height and average daily wither height was greater among calves receiving greater amounts of yeast. Lesmeister et al. (2004) speculated that increased structural growth in calves consuming greater amounts of yeast culture may have been a result of additional nutrients and energy from increased DMI.

Seymour et al., (1995) reported that calves fed yeast had less DMI than calves that did not receive yeast (Seymour et al., 1995). No effect of treatment ($P = 0.97$) was found on ADG. Nonetheless, these authors (Seymour et al., 1995) observed that yeast improved ADG from 12 to 25 d of age among calves fed colostrum, but yeast reduced ADG among calves fed a colostrum substitute (*colostrum source* × *yeast* = 0.04). Additionally, improvements in ADG were preceded by improvement in efficiencies of gain ($P = 0.03$)

among calves fed yeast during transition to dry feed (5 to 11 d of age). These authors (Seymour et al., 1995) speculated that yeast may have improved calves' acclimation of calves to a non-milk based diet. No differences were observed in body weight gain post-weaning. Overall, yeast did not affect performance.

Hill et al. (2009) supplemented calves with live yeast or MOS. Supplementation with yeast or MOS did not affect DMI ($P = 0.59$), CP intake ($P = 0.87$), NDF intake ($P = 0.92$), ADF intake ($P = 0.97$), feed efficiency ($P = 0.45$), ADG ($P = 0.38$), hip width ($P = 0.48$) or wither height ($P = 0.32$); however, final body weight was greater ($P < 0.01$) among Jersey calves that received either MOS or yeast compared to control. Hill et al. (2009) concluded from their data that supplementation with yeast or MOS to Jersey calves could be beneficial.

Galvão et al. (2005) evaluated effects of yeast on performance of calves with failure of passive transfer. Live yeast was added to grain (SC), milk replacer (SB), both (SCSB) or none (control). Calves fed SC prior to weaning had a greater ($P < 0.05$) grain DMI when compared to control and calves fed SB prior to weaning tended to have greater grain DMI ($P < 0.10$). Grain intake after weaning tended ($P < 0.10$) to be greater in calves fed SC. Calves fed SC had greater BW gain ($P < 0.02$) than control. Additionally, calves fed SB tended ($P < 0.10$) to have increased gains. Galvão et al. (2005) speculated that effects on body weights were from greater intake of grains, not from improved feed efficiency. Galvão et al. (2005) also reported an interaction between treatment and age of calves among BW gain and stated that SC and SB treatments had positive effects on weight gain early in life or prior to weaning.

Nocek et al. (2011) studied effects of supplementing early lactation dairy cattle with yeast culture and enzymatically hydrolyzed yeast. Nocek et al. (2011) fed cows no yeast, yeast culture (YC), and yeast culture with enzymatically hydrolyzed yeast (YC+EHY). Starting milk yields tended to be higher in cows receiving YC+EHY, and cows receiving YC and YC+EHY had greater milk secretions compared to controls during the first 77 days in milk (DIM; Nocek et al., 2011). Concentration of milk fat, lactose, urea-N, and BW and BCS were not affected ($P > 0.05$) by treatment. Milk protein concentration was greater ($P = 0.01$) among cows fed YC+EHY. Milk fat yield was greater ($P = 0.01$) among cows receiving YC and YC+EHY. Similarly, supplementation with YC+EHY increased milk protein yields (Nocek et al., 2011). Cows receiving YC and YC+EHY had greater ($P = 0.01$) 3.5% fat corrected milk and energy corrected milk when compared to control.

Bruno et al. (2009) evaluated effects of yeast fed to heat stressed cows. Treatments were control and yeast culture (*Saccharomyces cerevisiae*) and were assigned at calving. Exposure to heat stress was considered when the temperature-humidity index reached values above 72. Rectal temperatures of the cows were not affected by treatment with yeast culture. Dry matter intake was not different between treatments (Bruno et al., 2009). Energy corrected milk was not different between treatments. Milk yield was greater in cows receiving yeast culture. Milk fat was decreased ($P = 0.05$) in cows receiving yeast culture (Bruno et al., 2009). Decreased milk fat reduced ($P = 0.01$) estimates of dietary net energy for lactation among cows consuming yeast culture. Even though concentrations of milk components were not affected by treatment, total daily yields of proteins, solids-non-fat, and lactose increased ($P \leq 0.05$) in cows receiving diets

containing yeast culture. Feeding yeast culture during heat stress did not affect ($P \geq 0.18$) plasma glucose, NEFA, and 3-OH-butyrate concentrations (Bruno et al., 2009). Body condition scores, as well as blood energy metabolites and insulin, were not affected by treatment. Plasma urea N concentrations were less in cows fed yeast culture. These data (Bruno et al., 2009) demonstrate increased milk yields and increased solids-non-fat yield among cows receiving yeast when exposed to heat stress.

Yeast culture and components of yeast cells in many cases have no effect on performance. This has been noted by Cole et al. (1992), Dann et al. (1999), Lesmeister et al. (2004), Seymour et al. (1995), Nocek et al. (2011), and Bruno et al. (2009). Nonetheless, Williams et al. (1991) observed increased daily milk yields with yeast culture. Dann et al. (1999) reported that milk yield peaked earlier among cows fed yeast culture. Lesmeister et al. (2004) noted greater structural growth measurements when calves were fed yeast culture. Nocek et al. (2011) reported increased yield of milk components. Similar to Williams et al. (1991), Nocek et al. (2011) observed greater milk yields in cows fed yeast culture. Bruno et al. (2009) also noted increased milk yield in cows receiving yeast culture. Greater milk yield among beef cattle can increase weaning weights.

In the search for studies focused on the effects of yeast and yeast products, majority of the articles found focus on the response in dairy animals. In this literature review, Cole et al. (1992), Dann et al. (1999), Lesmeister et al. (2004), Seymour et al. (1995), Nocek et al. (2011), Bruno et al. (2009), Williams et al. (1991), Yuan et al. (2014), Campus-Granados et al. (2013), Spring et al. (2000), Franklin et al. (2005), Magalhães et al. (2008), Heinrichs et al. (2003), Seymour et al. (1995), Galvão et al.

(2005), Nocek et al. (2011), and Hill et al. (2009) all worked with dairy animals. The others focus on feedlot animals, Ponce et al. (2012), and Keyser et al. (2007). There is little data that focuses on beef cows.

It is clear that there is a lack of data on feeding yeast to beef cows.

Chapter II

Effects of enzymatically hydrolyzed yeast supplementation and supplementation frequency on immune parameters among periparturient beef cows and calves.

Abstract

Effects of enzymatically hydrolyzed yeast (EHY) and supplementation frequency (FREQ) on immune parameters among beef cows and calves were evaluated. Eighty multiparous (parity = 4.2 ± 0.3) cows were fed a common brome hay-based diet (CP = 8.0 ± 0.17%). Cows were blocked by expected calving date and stratified by BCS before random assignment of treatment. Beginning 88 ± 5 d prior to parturition, cows were provided 1 kg daily or 3 kg every 3 d of a soybean hull-based supplement (CP = 34.0%) that contained 0 or 3 g/kg EHY. The daily supplement was designed to meet ruminal N requirements. Cows were vaccinated against rotavirus at 62 and 48 ± 5 d prior to parturition. Cow sera, plasma, BW, and BCS were collected at 62, 48, 40, 24 and 14 d prior to parturition. At parturition, colostrum was milked from cows prior to feeding to calves and plasma, sera, and BW was collected from cows and calves. Subsequently, calf plasma was collected at 2 and 14 d after parturition. Calves were weighed on days 2, 14, and every 30 days until final weaning weight was recorded. Calf plasma IgG concentration increased (*quadratic* < 0.01) as age increased and passive transfer status among calves was 'excellent' (i.e. plasma IgG concentration was greater than 15 g/L among calves aged 2 d; APHIS, 2010). Nonetheless, plasma IgG was greater ($P = 0.03$) among calves born to cows supplemented EHY and FREQ had no effect on plasma IgG in calves. Despite differences among calf plasma IgG concentrations, there was no effect of EHY or FREQ on colostrum yield, colostrum concentration of IgG or calf intake of colostrum. Similarly, apparent efficiency of IgG absorption (AEA) and sera rotavirus neutralization titers (RNT) among calves aged 14 d was not affected by treatment ($P \geq 0.36$). Cow plasma IgG decreased (*quadratic* = 0.02) as cows neared parturition and were

not affected by EHY ($P = 0.56$) or FREQ ($P = 0.14$). We observed a quadratic ($P =$) increase in rotavirus neutralization titers in cow sera in response to vaccination, as expected. Sera rotavirus neutralization titers were not impacted by EHY ($P = 0.70$) or FREQ ($P = 0.42$). Cow BCS increased (*linear* < 0.01) over the entire pre-parturient period. Cow BCS tended to be effected by FREQ ($P = 0.06$) but was not affected by EHY ($P = 0.91$). Gestation length and calving ease scores were not affected by EHY ($P \geq 0.20$), FREQ ($P \geq 0.20$), or their interaction ($EHY \times FREQ = 0.15$). Calf BW increased with age (*linear* < 0.01) but calf BW was not affected by frequency ($P = 0.90$) or EHY ($P = 0.55$). Cow milk production was not affected by FREQ ($P = 0.23$) or EHY ($P = 0.59$). Similarly, milk far yield was not affected by FREQ ($P = 0.34$) or EHY ($P = 0.63$). Energy correct milk yield decreased (*quadratic* < 0.01) but was not affected by FREQ ($P = 0.25$) nor EHY ($P = 0.78$). Similarly, milk protein yield decreased (*quadratic* < 0.01) but was not affected by FREQ ($P = 0.13$) nor EHY ($P = 0.69$). Cow milk somatic cell count (SCC) was also not affected by FREQ ($P = 0.40$) or EHY ($P = 0.24$). In summary, EHY fed to gestating beef cows increased calf plasma IgG and FREQ tended to increase milk protein yield.

KEYWORDS: cattle, supplementation frequency, yeast

Introduction

Passive transfer of immunity occurs by intake and subsequent absorption of immunoglobulins in colostrum after birth. Passive transfer of immunity is important to the health of neonates and long-term performance of cattle. Failure of passive transfer may contribute to as many as 100,000 deaths among calves in the United States annually (APHIS 2010; NASS, 2016). Passive transfer of immunity occurs by intake and subsequent absorption of immunoglobulins in colostrum after birth. Yeast inclusion in feed can augment cattle immunity. Various types of yeast products have been included in research including live yeast, killed culture, hydrolyzed yeast cells, and components of yeast cell walls. Franklin et al. (2005) reported an increase in passive transfer of rotavirus neutralization titers (RNT) among calves from cows fed oligosaccharides derived from yeast cell walls (i.e. mannan oligosaccharides). A similar response has been reported among cattle fed enzymatically hydrolyzed yeast (EHY). Ponce et al. (2012) reported a greater proportion of control heifers were treated for BRD than heifers fed EHY. A majority of reports on effects of yeast on immune response in cattle are related to lactating dairy cattle, data are limited for effects of yeast on immune response of beef cattle. Effects of supplementation frequency (FREQ) and EHY supplementation to preparturient beef cows on calf performance and health was evaluated. Supplementation frequency and EHY supplementation were the 2 factors (i.e., treatments) that were evaluated in this experiment. The goal was to determine effects of treatment (i.e., supplementation frequency and EHY) on immunity and performance. A justification for the need for these data is that a large number of beef cattle are provided a protein supplement on a weekly or twice weekly basis instead of a daily basis, (citation) which is

different than how most dairy cattle are fed yeast (i.e., most dairy cattle are provided a total mixed ration daily). Additionally, relatively little data are available on effects of EHY in comparison to yeast cultures.

Materials & Methods

Animals, husbandry, and diet. All experimental procedures were approved (protocol no. 13-087A) by the South Dakota State University Institutional Animal Care and Use Committee. Eighty pregnant multiparous (parity = 4.2 ± 0.3) Angus and Simmental x Angus cows (initial BW = 653.3 ± 16.4 kg) were kept in a single paddock (4.98 ha), blocked by expected calving date and stratified by BCS (measured at 118 ± 0.6 d prepartum) prior to random assignment to 1 of 4 treatments. Beginning 93 ± 0.6 d prepartum, cows were fed grass hay (CP = 8.0 ± 0.17%; NDF = 59.9 ± 0.0%; ADF = 41.2 ± 0.0%; ADIA = 5.2 ± 0.4%) to ad libital and provided a protein supplement (Table 1) that contained 0 or 0.3% enzymatically hydrolyzed yeast and yeast cell metabolites (EHY; TruMax, Vi-COR Inc. Mason City, IA) daily or every 3 d. Hay was sampled via a forage probe (Penn State Forage Sampler, 2.86 cm dia. x 46 cm Round Shank). Two cores were removed from each bale prior to delivery to cows and composited for subsequent analyses. Cores were taken 5 cm apart on the wrapped side of the bale and directed towards the center of the bale. The probe was drilled into the bale approximately 30 cm. Cows were individually fed supplement in a double 5 stall individual feeding system and subsequently returned to the paddock. Cows received either 1.0-kg of protein supplement daily or 3.0-kg (as-fed basis) every 3 d so that cattle assigned EHY received either 3 g daily or 9 g every 3 d. Cows were provided supplement on the appropriate d at 1000 h. Each supplement was designed to meet ruminal N requirements (total or DIP?)

based on a. priori CP analysis of hay = 7.5 ± 0.1% and balanced to account for cold stress based on the average ambient temperature (-9°C) between December and March over the last 10 years. The average ambient temperature during this study was -13 ± 10.8°C. Microbial efficiency was adjusted to 10% (Lardy et al., 2009) for estimates of ruminal available N requirements (NRC; 2000).

At d 67 prior to calving, serum (10mL; BD Vacutainer tube, #366430; Becton Dickinson and Company, Franklin Lakes, NJ) and plasma (10 mL; BD Vacutainer tube, #366643; 1.8 mg EDTA/mL of blood estimated to be drawn). Body weight and BCS were recorded. Body condition score of cows was evaluated by 3 trained technicians after collection of blood samples on the same day. Cows received a vaccination of bovine rotavirus (serotypes G6 and G10), coronavirus, K99 E. coli bacterin, and Clostridium perfringens type C toxoid (Scourguard 4 kc; Zoetis, Kalamazoo, MI) on d 64 and d 42 prior to calving. Body weight, BCS, and blood samples were then taken every 14 d until parturition.

At parturition, cows and calves were weighed and blood samples were collected. Calving ease was measured on every cow (Albera et al., 2004). Prior to collection of colostrum, 2 ml of oxytocin was given to the cows and teats were prepared for milking. Colostrum was collected via a portable milking unit (# Z15667N; eNASCO, Fort Atkinson, WI). Colostrum was weighed, a sample collected and frozen for later analysis, and then total colostrum intake recorded by weight of colostrum consumed via bottle. After calving, cows were fed a diet consisting of corn silage (50% DM in diet) and ground alfalfa hay (50% DM in diet) and moved to a new common location (5 hectares). On 2 and 14 d of age calves were weighed and blood samples were collected for analysis

of rotavirus neutralization titers (RNT) and Immunoglobulin G (IgG). Calves were weighed every 30 d after birth until weaning to monitor calf performance. Cows were milked via a portable milking unit on 30, 90, and 150 d post calving for record of milk production performance. Milk was sent to Dairy Herd Improvement Association (DHIA) in Manhattan, KS, for a 6-channel analysis. Calves were separated from cows 12 hours prior to milking (Beal et al., 1990).

Feed sampling. Samples of supplement were collected daily (100 g/d). Replicate cores were collected from each hay bale delivered to cows with a forage probe (Penn State Forage Sampler, eNASCO) and composited. The forage probe was angled towards the center of the bale from the net wrapped side of the bale. The probe was driven into the bale about 0.305 m.

Blood sampling and analysis. Blood was collected via jugular venipuncture to allow collection of plasma (5.4 mg K_2EDTA) and sera. Plasma was harvested via centrifugation at 1,000 x g at 4°C for 15 mins. Prior to centrifugation, a small amount of blood was placed in 2 hematocrit tubes and then one end packed with clay. The hematocrit tubes were centrifuged and packed cell volume measured (was this analyzed?). Plasma samples were analyzed for IgG (E11-118; Beythl Laboratories, INC., Montgomery, TX), plasma urea nitrogen (PUN, QuantiChrom Urea Assay Kit; BioAssay Systems, Hayward, CA), and total proteins (Coomassie Protein Assay Kit #23200; Thermo Scientific, Rockford, IL).

Analysis of IgG in plasma required a dilution of the sample plasma to 1:250,000. Diluted samples were vortexed, then 100 µl was added to designated wells. Samples or standards were run in duplicate. Plate was covered and incubated at room temperature

(20-25 °C) for 1 hr. Plate was washed four times in a standard plate washer. Then 100 µl of anti-IgG detection antibody was added to each well. Again plate was covered and incubated at room temperature for 1 hr, after which plate was washed four times. Next, 100 µl of HRP (horseradish peroxidase) solution C was added to each well, plate was covered, and incubated at room temperature for 30 mins. Again, plate was washed four times. Next, 100 µl of 3,3',5,5'-tetramethylbenzidime (TMB) was added to each well, plate was incubated for an additional 30 mins in the dark at room temperature. Colorimetric reaction of TMB was stopped upon completion of 30 min incubation with 100 µl of dilute sulfuric acid. Plates were then read on a plate reader at 450 nm. Same procedure was utilized for colostrum samples as well except colostrum samples were diluted to 1:750,000.

Plasma urea nitrogen (PUN) was analyzed by plating undiluted samples on a 96-well plate then adding 200 µl of combined reagents of A (<0.4% o-phthalaldehyde and 10% sulfuric acid) and B (<0.8% boric acid and 22% sulfuric acid). Plates were incubated 20 mins at room temperature then read on a plate reader at 520 nm.

Plasma proteins were analyzed by coomassie (Bradford) protein analysis kit (Thermo Scientific). Samples were diluted to 1:100 dilution then 5 µl was added to a 96-well plate. Added to each well was 250 µl of Coomassie Reagent (coomassie G-250 dye, methanol, phosphoric acid and solubilizing agents in water). Plates were then moved to the plate reader and mixed using plate shaker function for 30 sec. Plates were incubated for 10 min at room temperature the read at 595 nm on plate reader.

Sera was analyzed for rotavirus neutralization titers (Besser et al. 1988, Brüssow et al. 1990). Briefly, serum was heat incubated at 56°C for 30 min to inactivate

complement. Rotavirus and serum were then combined in media. Cells (MARC145) were grown in a monolayer at 37°C in media. Cells were removed using trypsin and split into fetal bovine serum (FBS) media. Cells were then allowed to be infected with virus and sera mixture on a 96-well plate in decreasing dilution and incubated at 37°C for 24 h. Cells were then fixed with 80 % acetone for 30 mins at room temperature. Acetone was removed and cells were allowed to dry. The cell layer was wet with phosphate-buffered saline (PBS) then at least 30 µl of fluorescein isothiocyanate was added to each well and incubated overnight (12 h) at 4°C. Endpoint neutralization titers were at highest serum dilution that resulted in a 90% or greater reduction in fluorescent foci (Besser et al. 1988; Brüssow, et al. 1990).

Colostrum and milk sampling and analysis. Total colostral yield was measured gravimetrically after mechanical milking. To ensure complete collection of colostrum and milk, 2 ml (40 United States Pharmacopeia unit, USP) of oxytocin was given to each cow prior to milking. Colostrum density was measured after correction for temperature and immunoglobulin content estimated (Colostrometer, Biogenics, Florence, OR). An aliquot of colostrum (45 ml) was frozen (-20°C) and retained for IgG analysis. Subsequently, colostrum intake was recorded by weight of total consumption by calf.

Calculations. Apparent efficiency of IgG absorption AEA was calculated (Arthington, et al., 2000) as:

$$\text{AEA, \%} = \frac{[\text{plasma IgG } \left(\frac{\text{g}}{\text{L}}\right) \times \text{plasma volume (L)} \times 100]}{\text{IgG intake (g)}}$$

Plasma volume was estimated as 8.9% of calf BW at parturition (Quigley et al. 1998). Colostrum density was calculated by the following equation: $\text{Colostrum density} = 1180 \times \text{specific gravity} - 1172$ (Quigley et al., 1994)

Statistical analysis. Data were analyzed as a 2 x 2 factorial treatment design with the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). The model included EHY, FREQ, EHY x FREQ with block as a random effect for non-repeated measures. For repeated measures the model included fixed effects of EHY, FREQ, d and interactions between all terms. Variables with uneven spacing used covariance structure of SP(POW). Variables with even spacing used covariance structure of compound symmetry. Calf gender was used as a covariate for analysis of birth weight and parity was a covariate for analysis of immune variables and cow milk yields. Kenward and Roger (1997) generalized linear adjustment used for calculating denominator degrees of freedom.

Results

Immunity. There was no interaction of frequency of supplementation and EHY ($P = 0.34$). As expected, cow rotavirus neutralization titers (RNT) increased ($Day < 0.01$) in response to vaccination (2450 dilution; $d < 0.01$). Neither frequency of supplementation ($P = 0.46$; Figure 1) nor EHY ($P = 0.72$; Figure 2) affected RNT. Plasma IgG decreased ($quadratic = 0.02$) as cows neared parturition (Figure 3) but plasma IgG in cows was not affected by supplementation frequency ($P = 0.14$; Figure 3) or EHY ($P = 0.56$; Figure 4). Colostrum yield ($P \geq 0.33$), Ig estimates ($P > 0.15$), temperature ($P \geq 0.30$), density ($P \geq 0.16$), and IgG levels ($P \geq 0.12$) were not affected by EHY, frequency or EHY × frequency (Table 3). Average colostrum temperature was 15 ± 0.6 °C. Passive transfer among calves was ‘excellent’(i.e. plasma IgG was greater than 15g/L among calves aged 2 d; APHIS, 2010). Plasma IgG was greater ($P = 0.03$) among 2-d old calves born to cows supplemented EHY (35.97 ± 3.34, Figure 5) but frequency had no effect ($P = 0.19$) on plasma IgG in calves (35.97 ± 3.45, Figure 6). Similarly, RNT among calves aged 14

d was not affected by EHY (P = 0.36) or FREQ (P = 0.40, Table 3, Figure 7). Intake of colostrum by calves was not affected by treatment (P > 0.63, Table 3). Apparent efficiency of IgG absorption from colostrum in calves from cows fed EHY and 3-d was numerically greater (*EHY* × *FREQ* = 0.80, Figure 8). Additionally, birth weight was less (P = 0.05) among calves from cows fed EHY.

Performance. Cow BCS increased (*linear* < 0.01) over the entire pre-parturient period and had a tendency to be less (P = 0.06, Figure 9) among cows supplemented each 3 d, but cow BCS was not affected by EHY (P = 0.91, Figure 10). Gestation length (283 ± 1.3 d) and calving ease, were not affected by EHY ($P \geq 0.20$), FREQ ($P \geq 0.20$), or their interaction (*EHY x FREQ* = 0.15, Table 3). A majority of cattle calved without assistance (n = 78) that resulted a small calving ease score (1.0 ± 0.02), and calving ease was not affected by EHY (P = 0.20) or FREQ (P = 0.20). Calf BW increased with age (*linear* < 0.01) but calf BW was not affected by FREQ (P = 0.90, Figure 11) or EHY (P = 0.55, Figure 12). Cow milk production was not affected by FREQ (P = 0.23, Figure 13) nor EHY (P = 0.59, Figure 14). Similarly, cow milk fat yield was not affected by FREQ (P = 0.34, Figure 15) nor EHY (P = 0.63, Figure 16). Energy correct milk yield decreased (*quadratic* < 0.01) but was affected neither by FREQ (P = 0.25, Figure 17) nor EHY (P = 0.78, Figure 18). Similarly, milk protein yield decreased (*quadratic* < 0.01) but was not affected by FREQ (P = 0.13, Figure 19) nor EHY (P = 0.69, Figure 20). Cow milk somatic cell count (SCC) was also not affected by FREQ (P = 0.40, Figure 21) or EHY (P = 0.24, Figure 22).

Discussion

Rotavirus neutralization titers in cows increased with vaccination. Titer levels were lowest prior to vaccination on d 64 and were greatest after a second vaccination on d 42. The immune system responds to vaccinations and each subsequent vaccination with increased immune cells and increased production of antibodies such as IgM and IgG (Mak et al., 2014). The body's natural immune response to vaccination with increased IgG levels could explain the quadratic increase in IgG by d. Supplementation with EHY did effect cattle immune system, as evidenced by a lack of response among RNT to EHY. Additionally, colotrum IgG levels, and immunoglobulin levels were not affected by EHY.

Apparent passive transfer of immunity among calves was 'excellent' (APHIS, 2010; i.e. plasma IgG > 15 g/L on d 2 of age). As expected, levels of IgG in calves were near zero at parturition. Calves do not absorb immunoglobulins through the placenta during gestation and only absorb immunoglobulins through the gut after consumption of colostrum. At 2 d of age, IgG in calf blood was greatest. Concentrations of IgG in calf blood decreased over time. Half-life of immunoglobulin IgG in cattle is on average 15.7 d (Nansen and Nielsen, 1966). This explains the decreases in IgG in calves at 14 d of age. Contrary to IgG results in calves, EHY did not affect RNT in calves. Supplementation with EHY does not directly impact the immune cells and increase production of immunoglobulins. Even though EHY had no effect on intake of colostrum, there was a numerical increase of AEA. Birth weights of calves from cows fed EHY were less which would result in estimates of plasma volume to be less.

Changes in plasma IgG in calves from cows supplemented with EHY could help to decrease loss of calves prior to weaning. As stated earlier, nearly 100,000 calves die

each year from possible failure of passive transfer of immunity. If addition of EHY to supplementation strategies to gestating beef cattle increases plasma IgG in calves, it is possible that there could be fewer deaths to calves. Calves in this study received 'excellent' passive transfer of immunity; however, it is possible that addition of EHY could provide benefit to calves that have lesser concentrations of plasma IgG. This possibility can only be positively concluded with a research study testing supplementation of EHY on cattle limited in passive transfer of immunity.

Cows supplemented with EHY or on different supplementation strategies had little effect on their performance. Addition of EHY to supplement of gestating beef cows was a small component of diet. Cow diet was balanced to account for cold stress, which justifies no loss of BCS or BW during feeding period. Cow BCS increased by less than a tenth of a BCS over pre-parturient period. Each BCS correlates to approximately 75-100 lbs (Eversole, 2009). In this study, cows increased a tenth of a BSC or roughly 7.4 to 10 lbs. This increase is not likely biologically significant and could be technician variation in BCS. Calf BW was not affected by addition of EHY to cow supplementation. Calves did not directly consume EHY. While EHY can impact apparent passive immunity transfer, subsequent effects of EHY on calf performance were not different than control.

Conclusion

Supplementing gestating beef cows with EHY resulted in an increase in IgG levels in their offspring at 2 d of age. When passive transfer of immunity is limited increases in plasma IgG may enhances immunity. However, measurements of factors leading to increased IgG levels do not differ among treatments. Increases in plasma IgG concentration were likely related to lesser birth weight and subsequently smaller plasma

volumes among calves from cows provided EHY. Supplementation of EHY to gestating beef cows had no effect on immune and colostrum parameters of cows.

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Tables & Figures

Table 1. Composition of supplements fed to preparturient cows (% of DM)

Ingredient	Dietary treatment	
	Control	EHY
Soybean hulls	49.5	49.3
Soybean meal	48.4	48.2
Urea	2.2	2.2
Enzymatically hydrolyzed yeast[1]	--	0.3

[1]TruMAX; Vi-COR Inc., Mason City, IA

Table 2. Chemical composition of supplements fed to preparturient cows

Nutrient	Supplement content, % of DM
DM	87.8
NDF	37.4
ADF	29.1
CP	34.0

Table 3. **Effect of enzymatically hydrolyzed yeast (EHY) supplementation and supplementation frequency (FREQ) on parturition and passive transfer of immunity.**

	Daily		3 d			Contrast		
Item	EHY	Control	EHY	Control	SEM	EHY	FREQ	EHY x FREQ
n	18	20	19	19				
Gestation length, d	283	283	281	284	1.27	0.30	0.43	0.33
Calving ease[2]	0.999	1.099	1.004	0.999	0.037	0.20	0.20	0.15
Calf plasma rotavirus NT[1]	3929	3028	4040	3886	1.19	0.36	0.40	0.50
Colostrum produced, kg	2.70	2.12	2.57	2.26	0.79	0.33	0.99	0.77
Colostrum Ig	84	106	87	72	20.7	0.77	0.21	0.15
Colostrum temp, °C	15.7	13.3	16.2	14.8	3.0	0.30	0.58	0.77
Colostrum IgG, g/L	121	74	106	122	34.4	0.44	0.41	0.12
Colostrum density, g/ml	1.06	1.08	1.07	1.05	0.017	0.83	0.24	0.16
Colostrum intake, kg	2.10	1.74	1.85	1.89	0.57	0.63	0.87	0.56
Apparent efficiency of absorption, %[3]	54.8	49.5	61.6	54.0	20.4	0.59	0.63	0.92

[1] Neutralization titers; Analyzed as log transformation
[2] Calving ease scores were collected on all calves, using a 1 to 3 scale (1 = unassisted, 2 = moderate assistance, 3 = difficult delivery).
[3] Calculated as AEA = [plasma IgG (grams per liter) x plasma volume (liters) x 100]/IgG intake (grams)

Figure 1. Effect of supplementation frequency (FREQ) on cow rotavirus neutralization titer levels. SEM = 1.15. (*FREQ* = 0.46). (*FREQ x d* = 0.38).

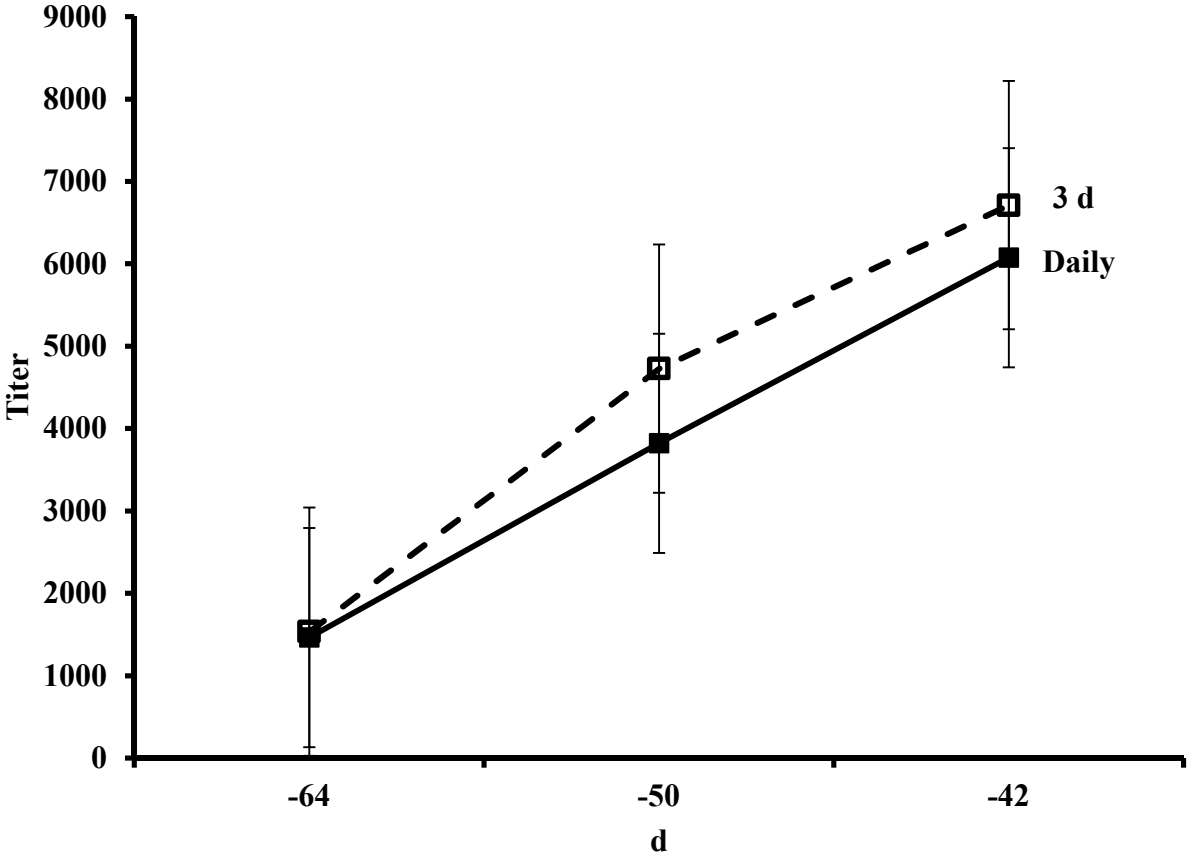


Figure 2. Effect of supplemental enzymatically hydrolyzed yeast (EHY) on cow rotavirus neutralization titer level. SEM = 1.15. (*EHY* = 0.72). (*EHY x d* = 0.32).

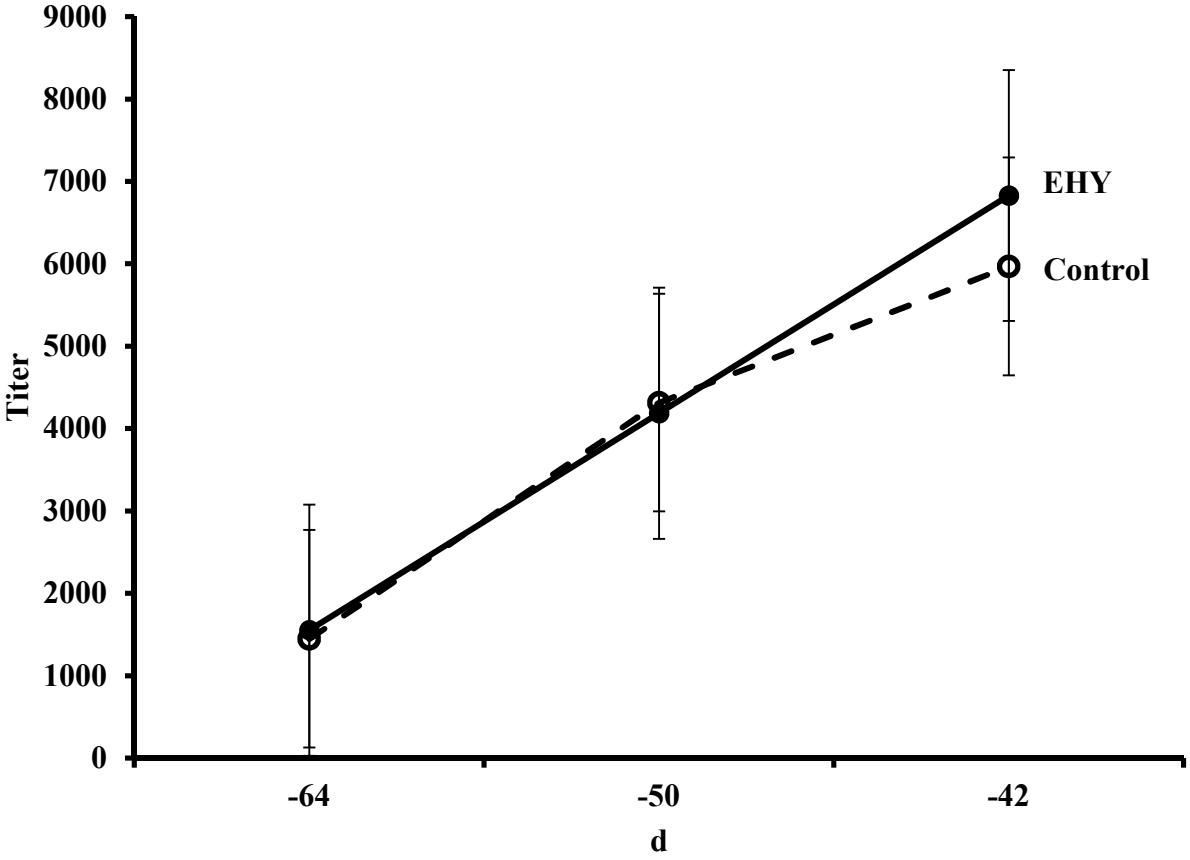


Figure 3. Effect of supplementation frequency (FREQ) on cow plasma IgG concentration. SEM = 1.09. (*FREQ* = 0.14). (*FREQ x d* = 0.72).

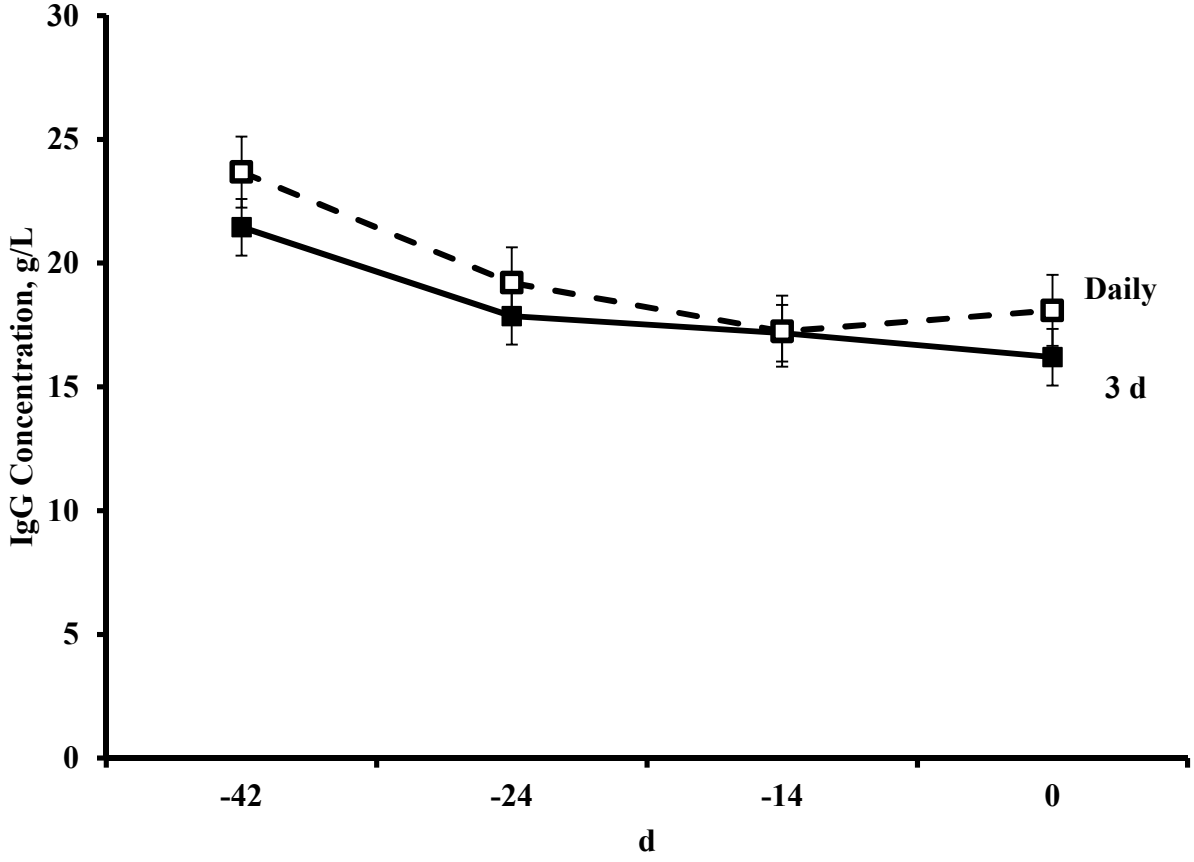


Figure 4. Effect of enzymatically hydrolyzed yeast (EHY) supplementation on cow plasma IgG concentration. SEM = 1.11. (*EHY* = 0.56). (*EHY x d* = 0.63).

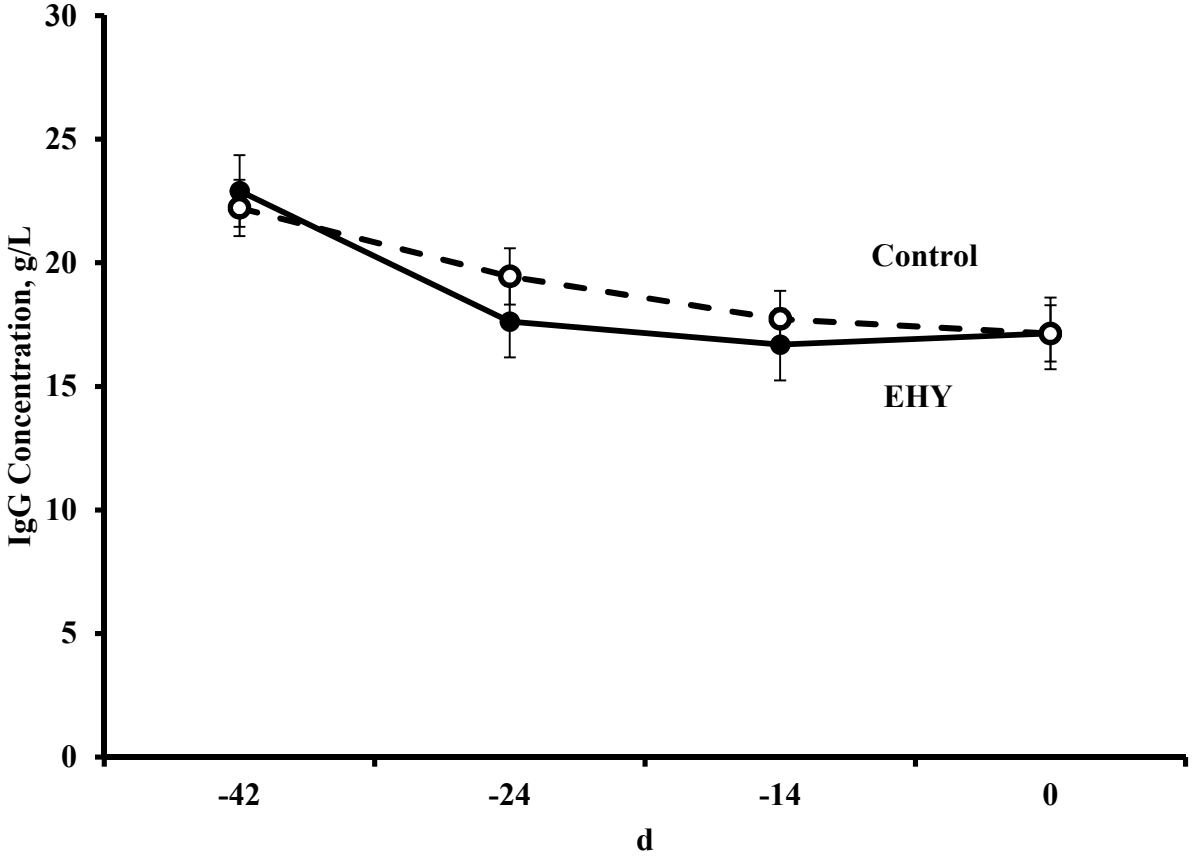


Figure 5. Effect of enzymatically hydrolyzed yeast (EHY) supplementation to gestating cows on calf plasma IgG concentration. SEM = 3.34. (*EHY* = 0.03). (*EHY x d* = 0.73).

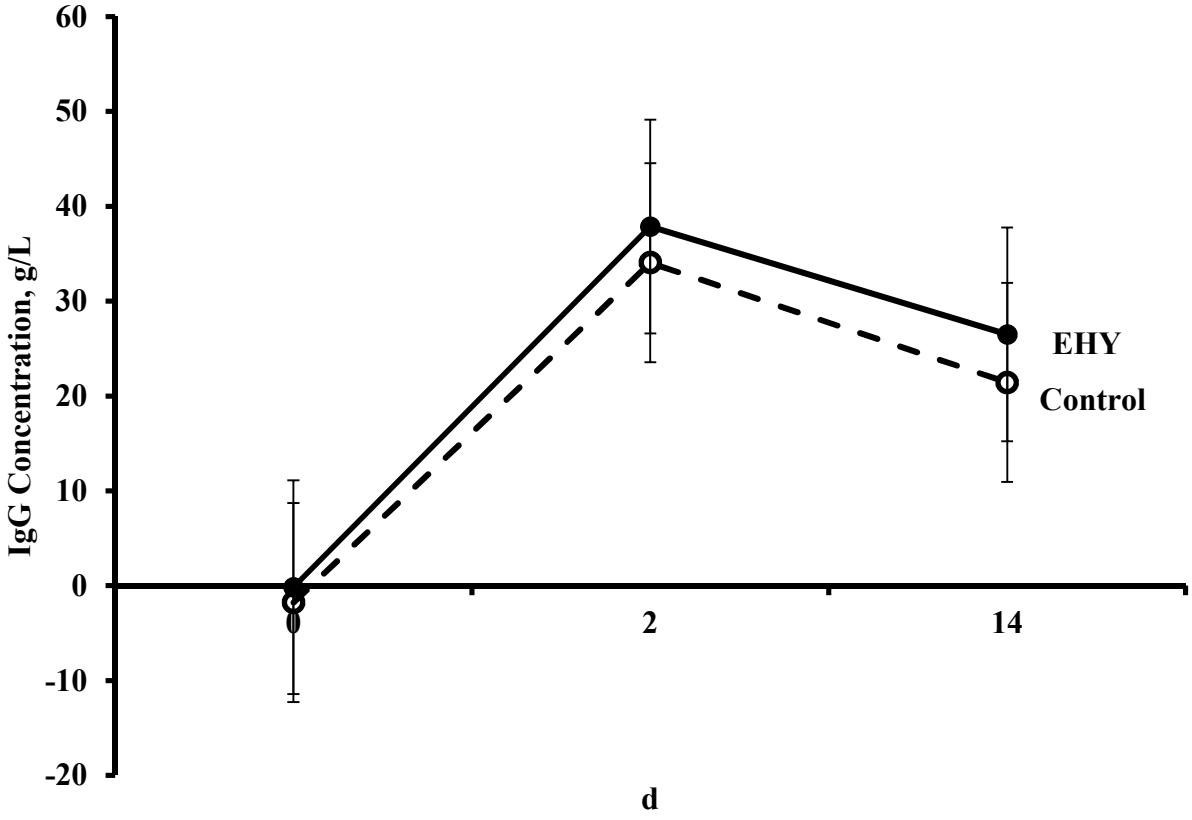


Figure 6. Effect of supplement frequency (FREQ) to gestating cows on calf plasma IgG concentration. SEM = 3.45. (*FREQ* = 0.19). (*FREQ x d* = 0.32).

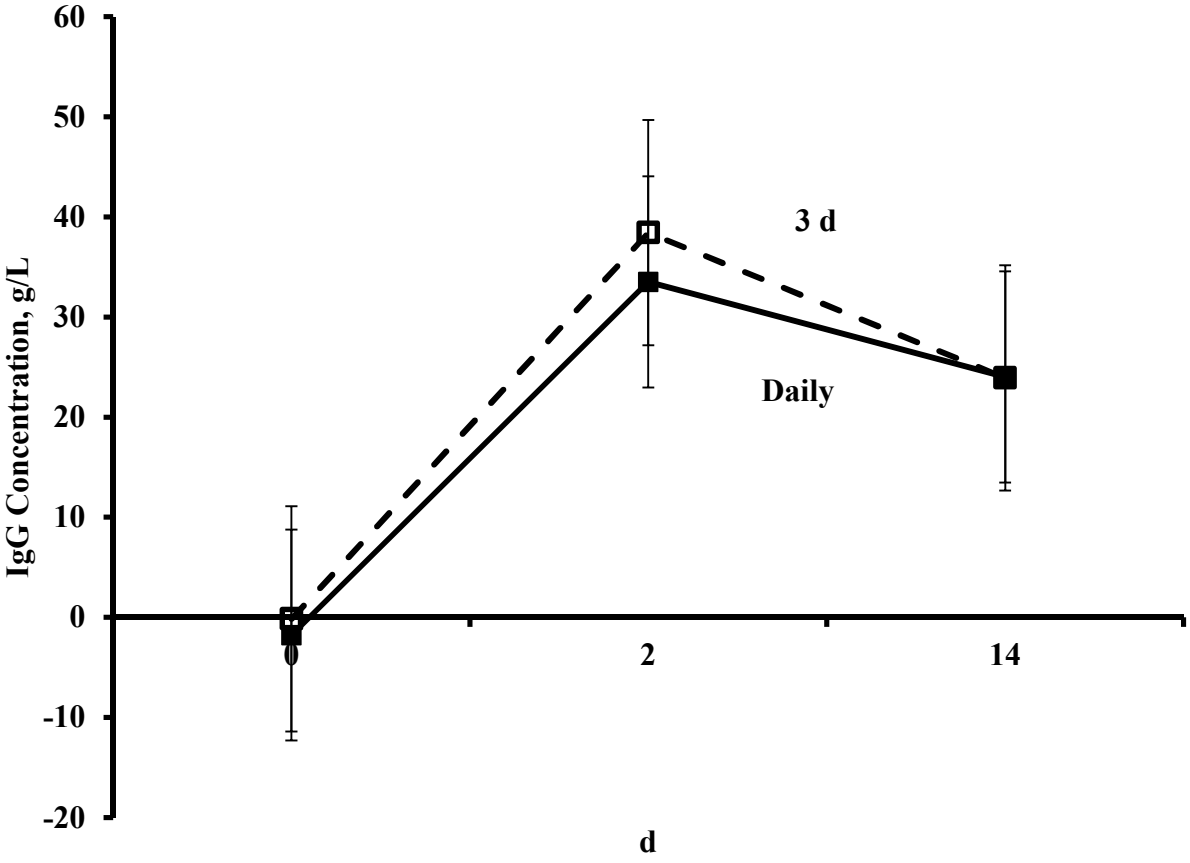


Figure 7. Sera rotavirus neutralization titer in calves born from cows provided a protein supplement daily (-d) or every 3 d (-3d) with (EHY) or without (Con) an enzymatically hydrolyzed yeast cell walls and yeast cell metabolites. [1]Means of $\log_{10}$ of the greatest dilution with neutralized rotavirus.

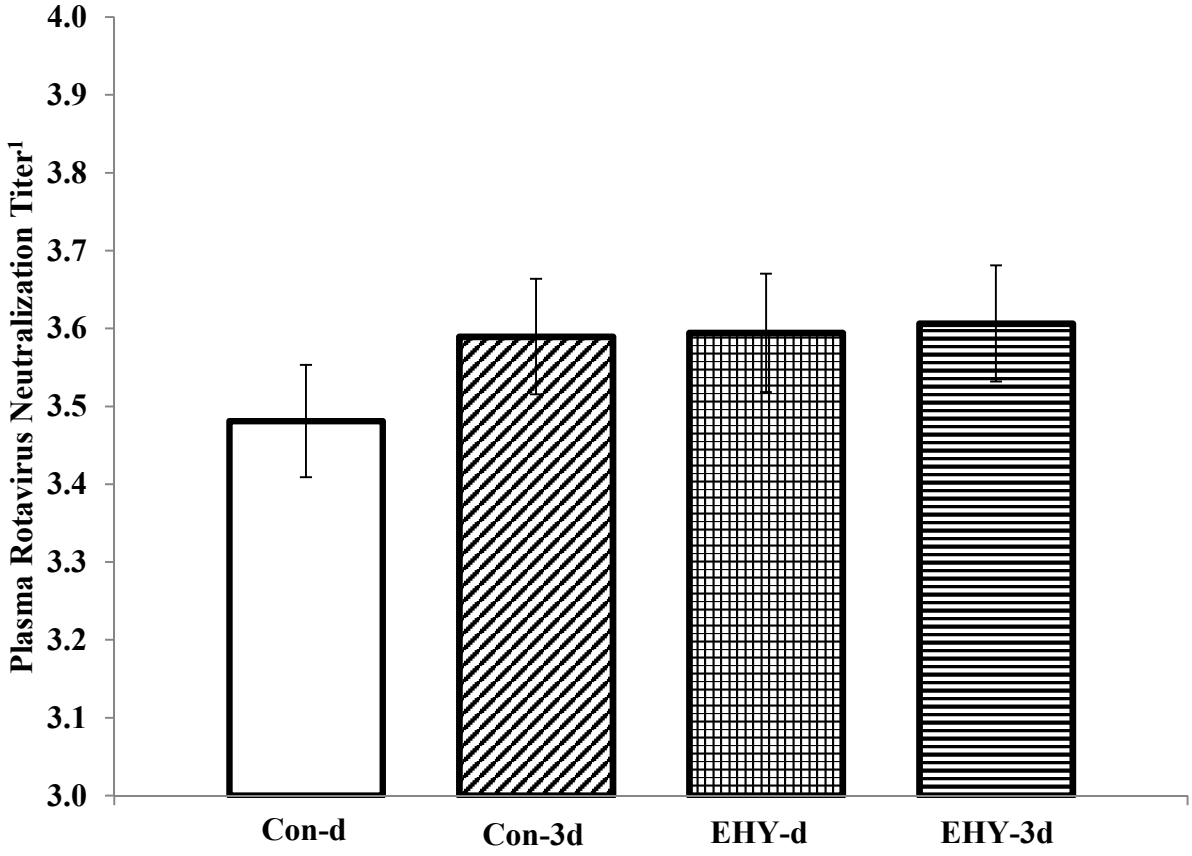


Figure 8. Apparent efficiency of IgG absorption in calves born from cows provided a protein supplement daily (-d) or every 3 d (-3d) with (EHY) or without (Con) an enzymatically hydrolyzed yeast cell walls and yeast cell metabolites product.

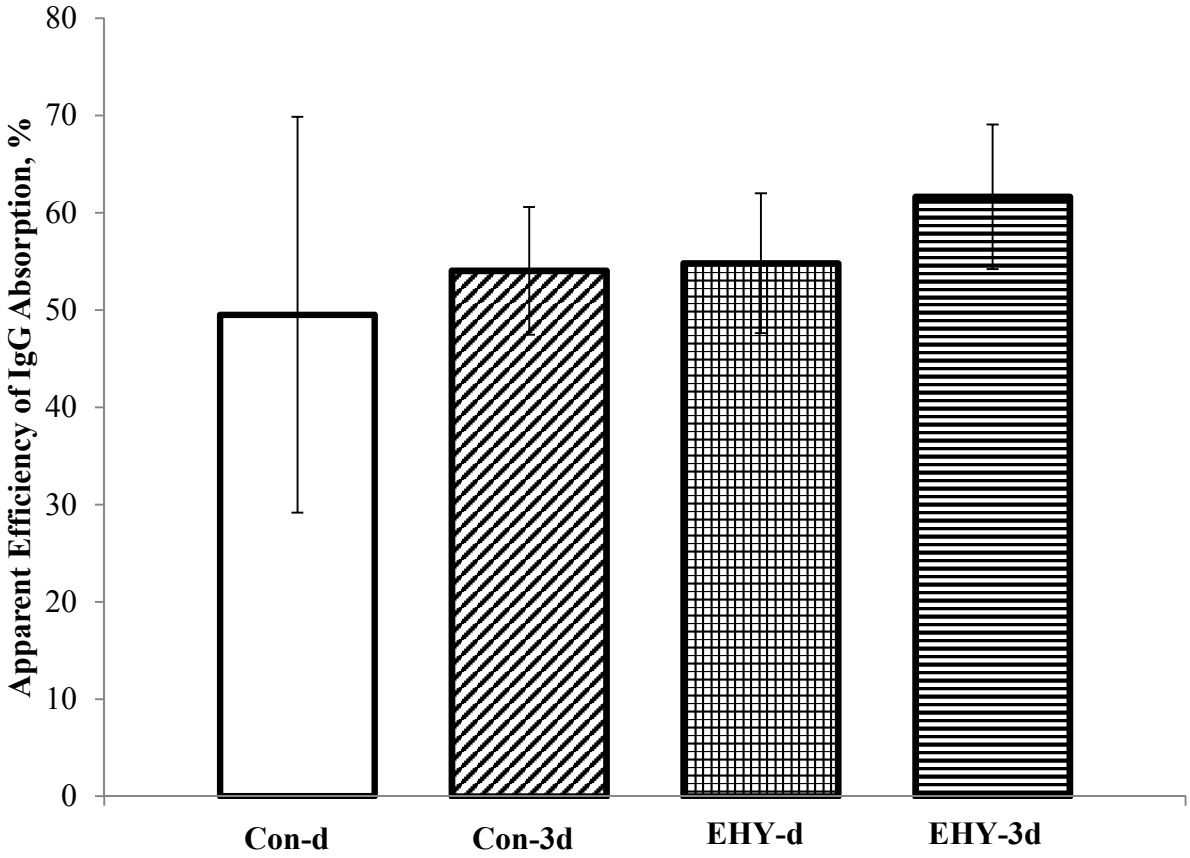


Figure 9. Effect of supplementation frequency (FREQ) on cow body condition score (BCS). SEM = 0.10. (*FREQ* = 0.06). (*FREQ x d* = 0.23).

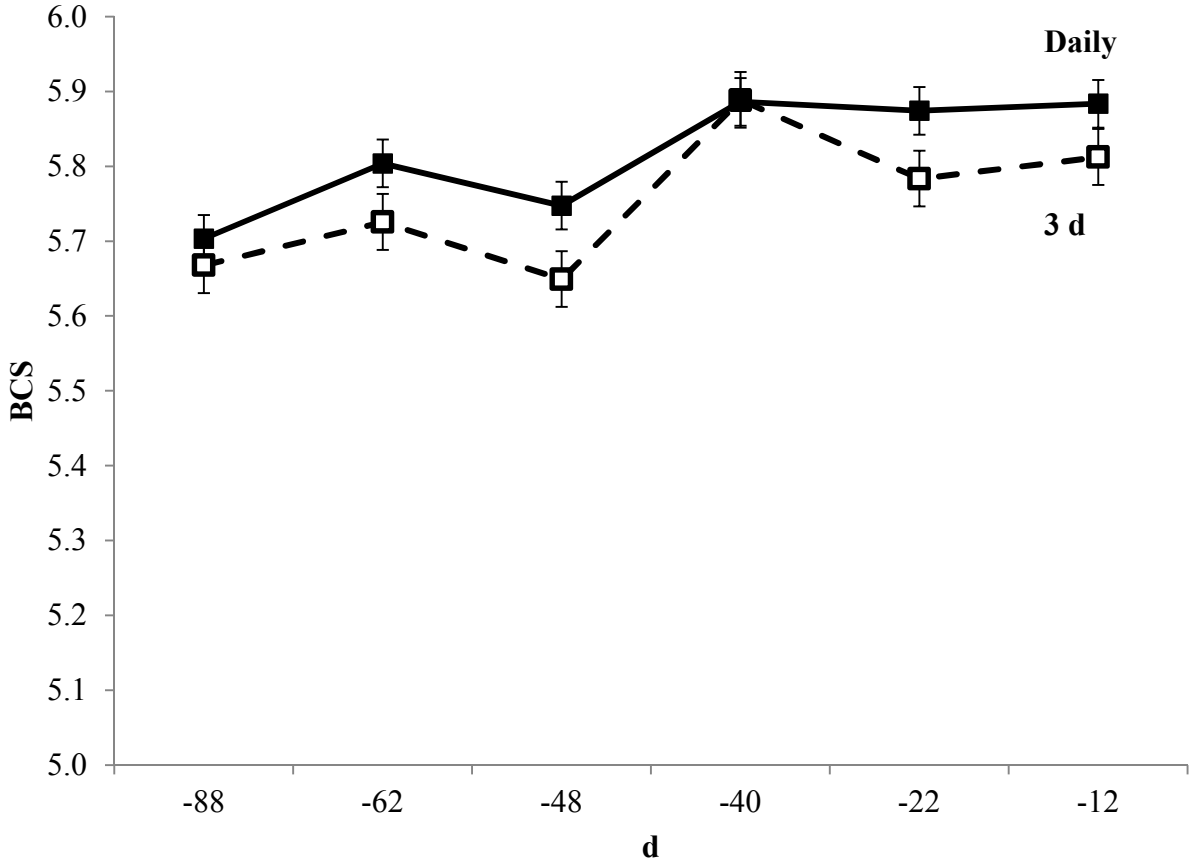


Figure 10. Effect of supplemental enzymatically hydrolyzed yeast (EHY) on cow body condition score (BCS). SEM = 0.10. (*EHY* = 0.91). (*EHY x d* = 0.58).

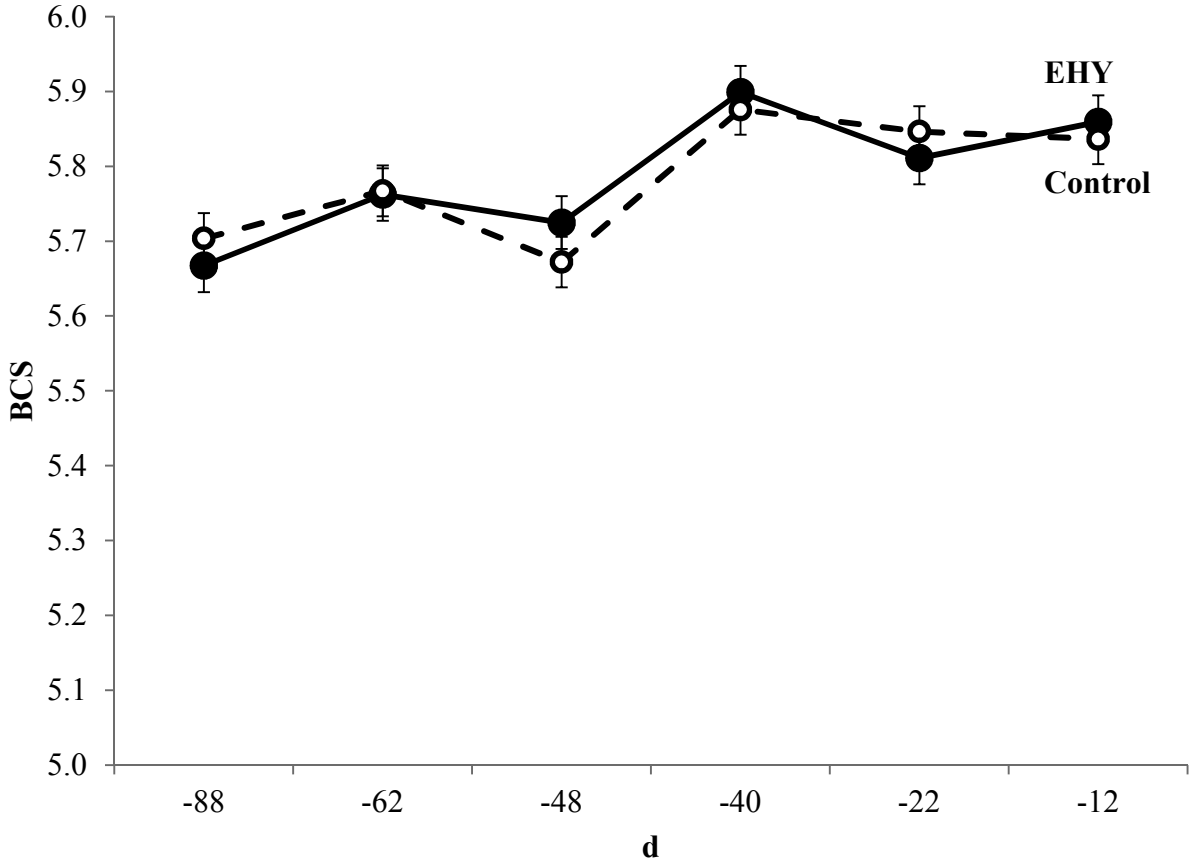


Figure 11. Effect of supplementation frequency (FREQ) on calf body weight (BW). SEM = 3.40. (*FREQ* = 0.90). (*FREQ x d* = 0.83).

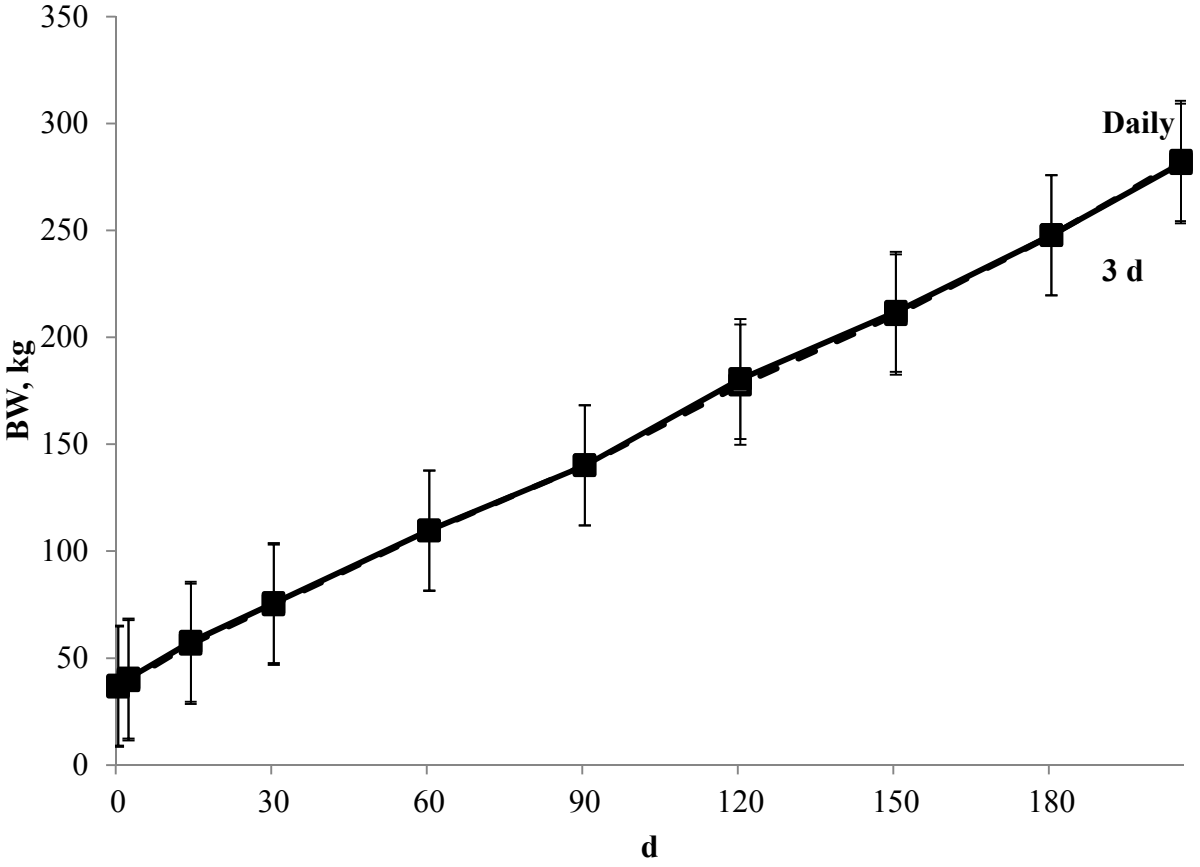


Figure 12. Effect of supplemental enzymatically hydrolyzed yeast (EHY) on calf body weight (BW). SEM = 3.45. (*EHY* = 0.55). (*EHY x d* = 0.25).

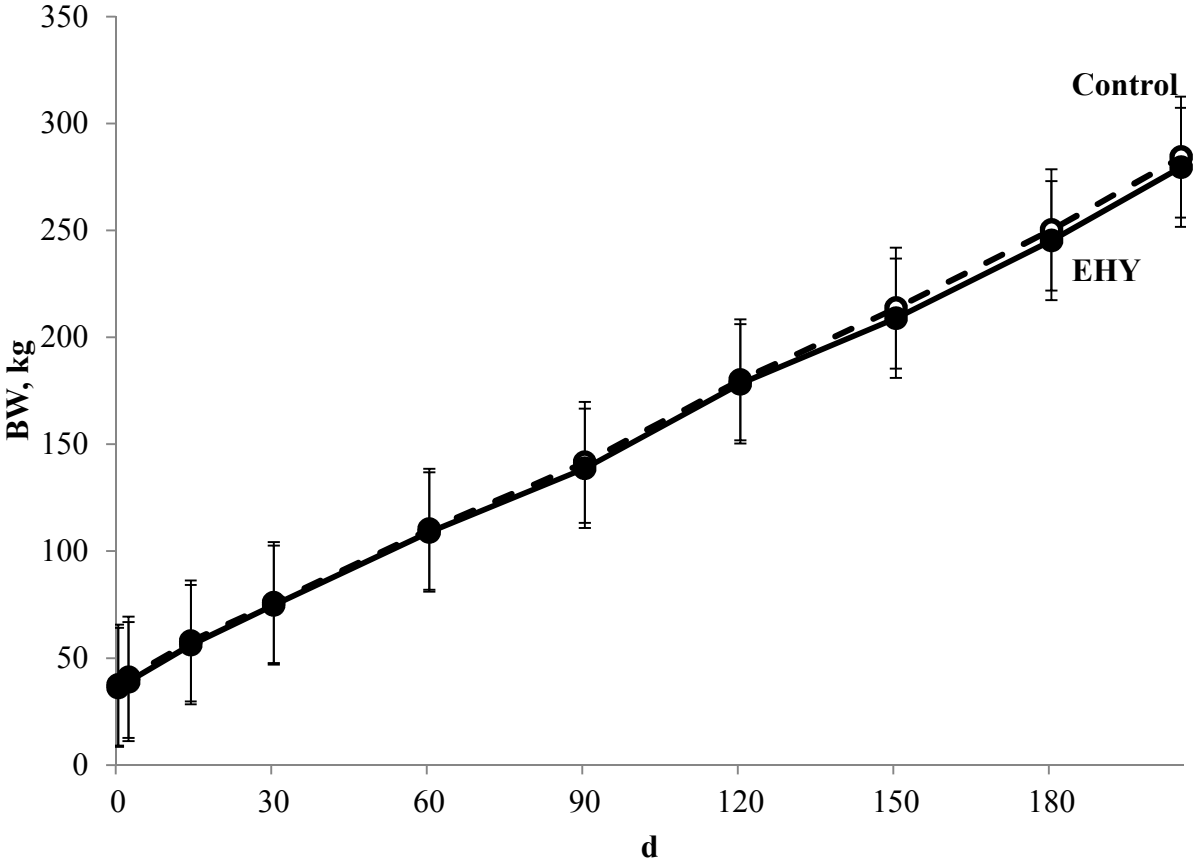


Figure 13. Effect of supplementation frequency (FREQ) on cow milk production. SEM = 0.2. (*FREQ* = 0.23). (*FREQ x d* = 0.70).

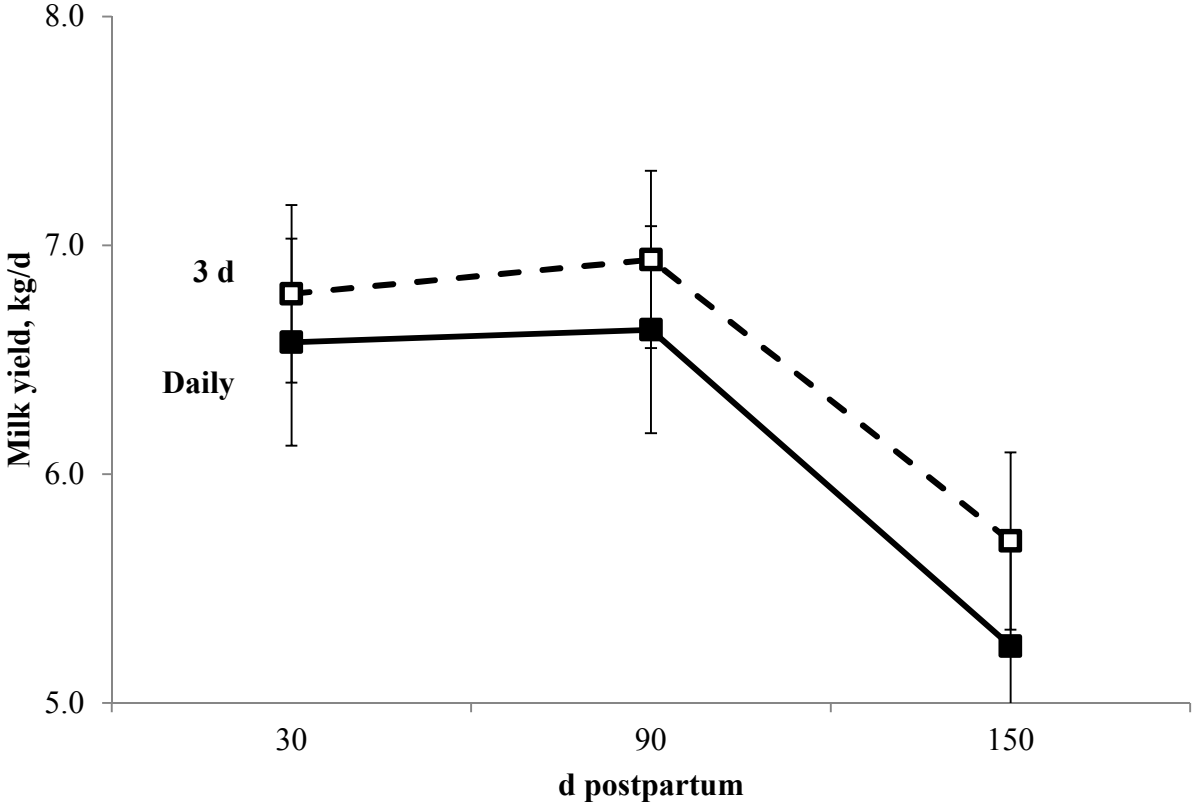


Figure 14. Effect of supplemental enzymatically hydrolyzed yeast (EHY) on cow milk production. SEM = 0.2. (*EHY* = 0.59). (*EHY x d* = 0.41).

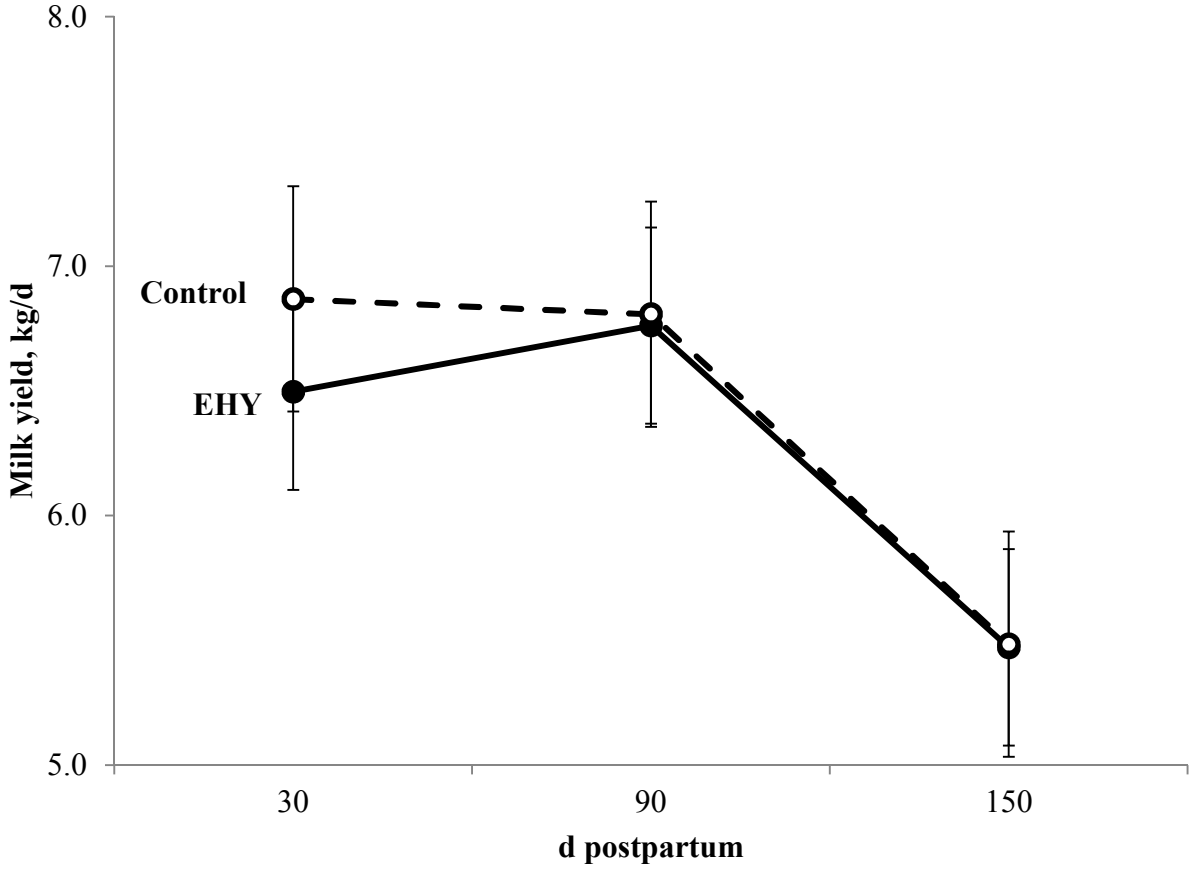


Figure 15. Effect of supplementation frequency (FREQ) on cow milk fat yield. SEM = 13.7. (*FREQ* = 0.34). (*FREQ x d* = 0.70).

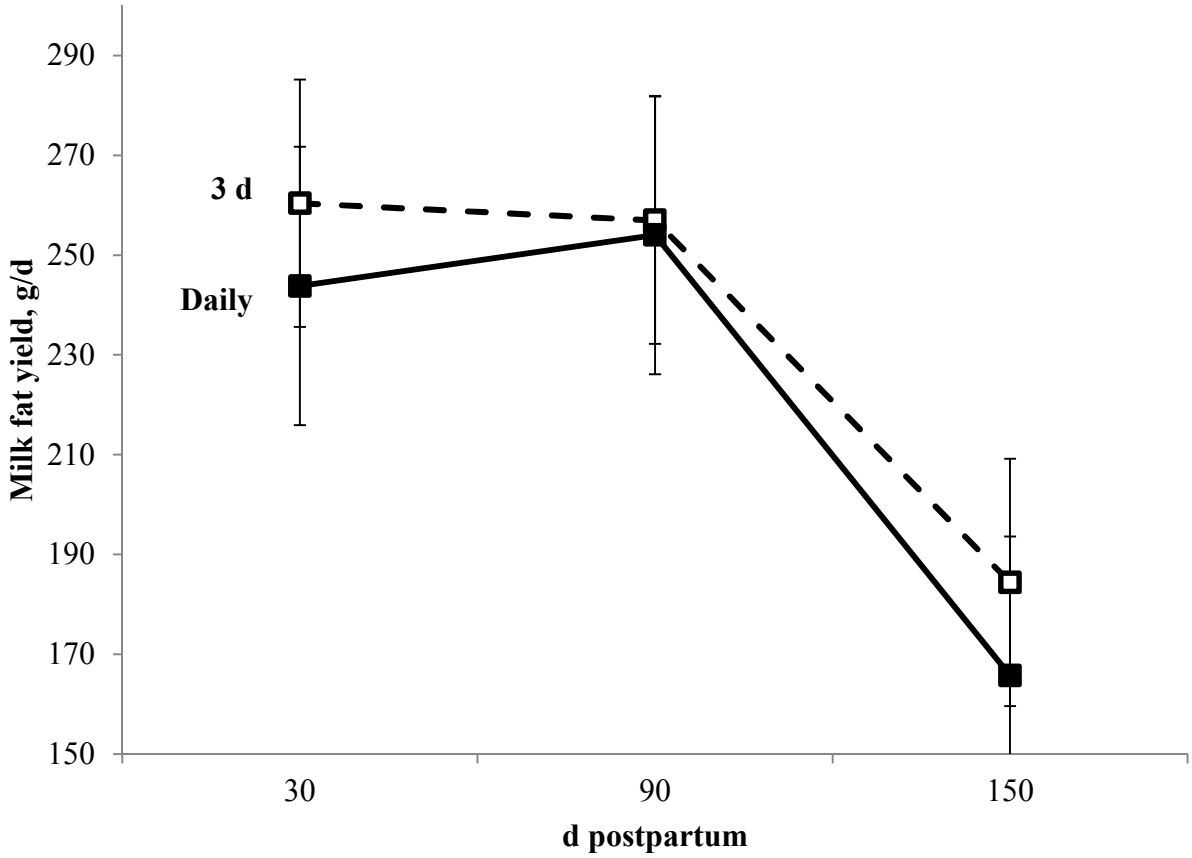


Figure 16. Effect of supplemental enzymatically hydrolyzed yeast (EHY) on cow milk fat yield. SEM = 13.7. (*EHY* = 0.63). (*EHY x d* = 0.07).

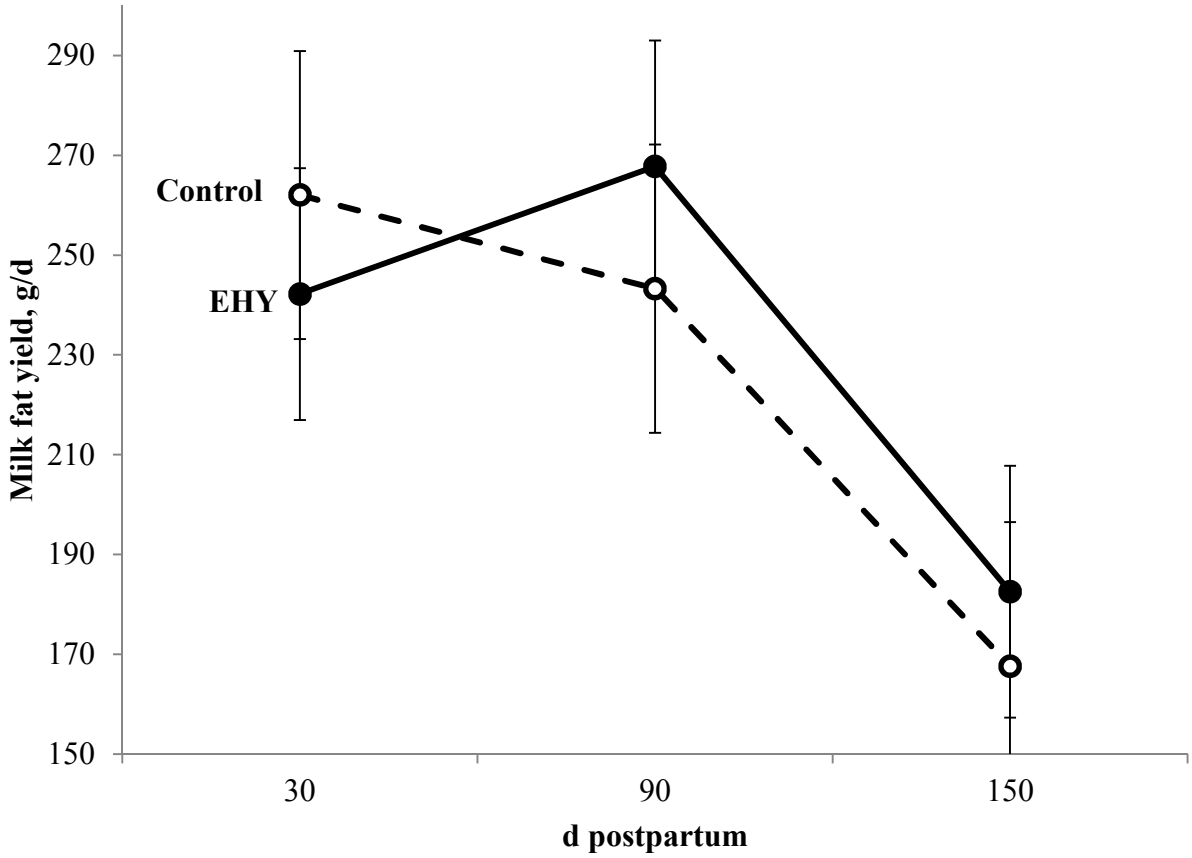


Figure 17. Effect of supplementation frequency (FREQ) on energy corrected milk (ECM) yield. SEM = -0.2. (*FREQ* = 0.25). (*FREQ x d* = 0.80).

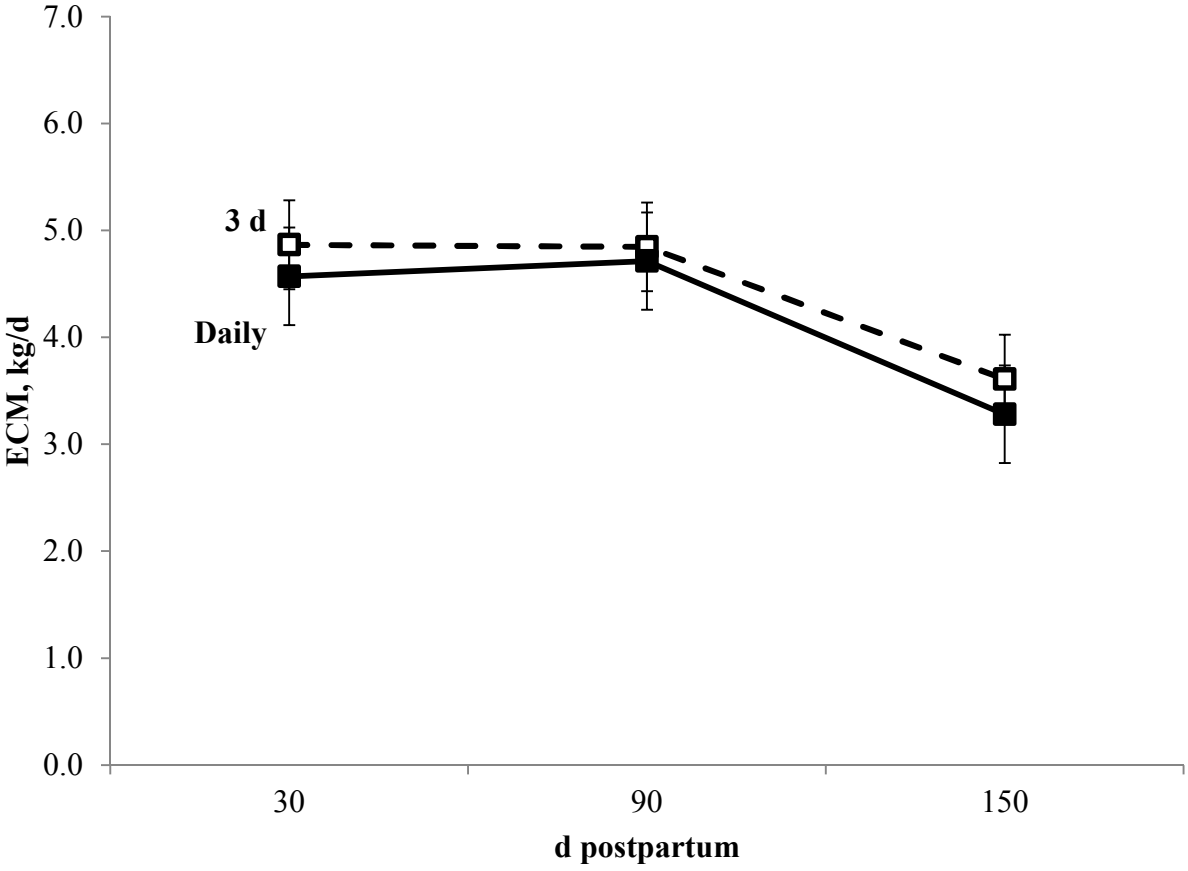


Figure 18. Effect of supplemental enzymatically hydrolyzed yeast (EHY) on energy corrected milk (ECM) yield. SEM = -0.2. (*EHY* = 0.78). (*EHY x d* = 0.10).

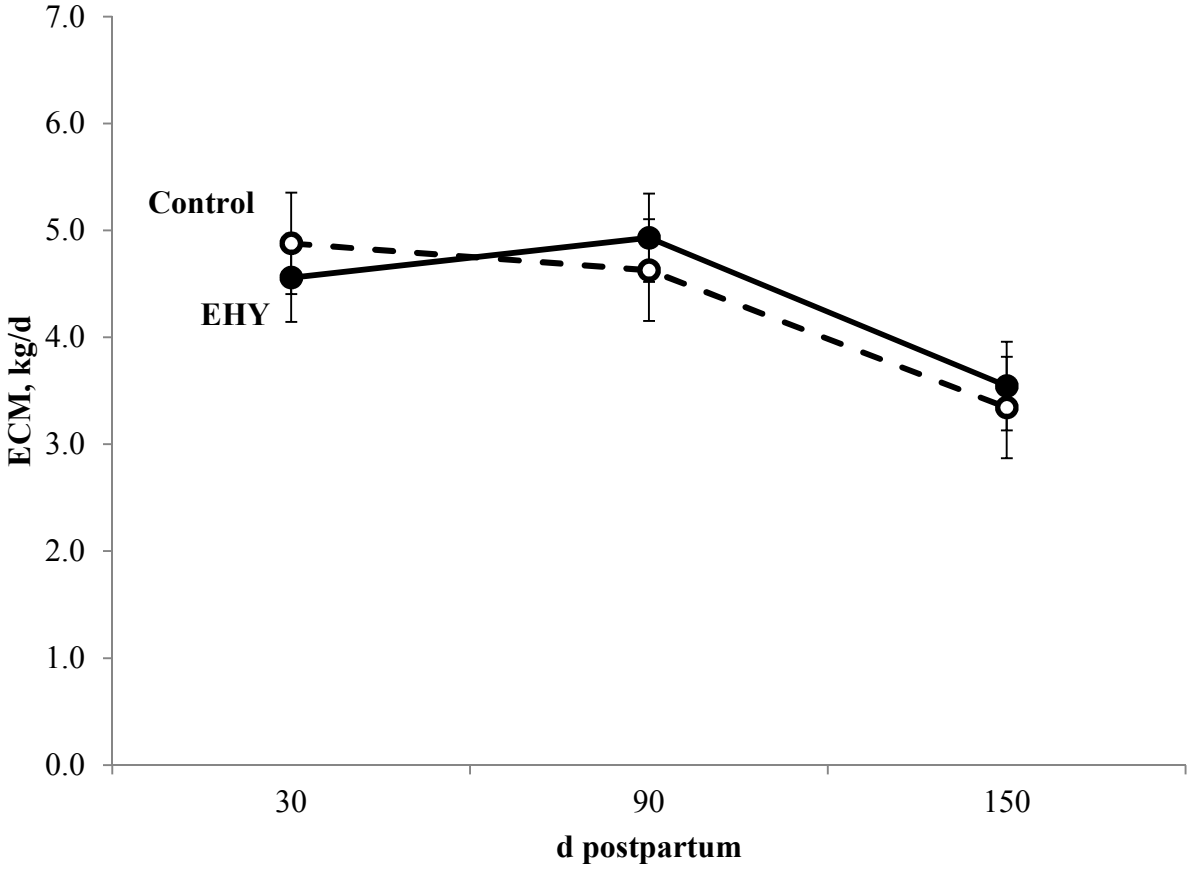


Figure 19. Effect of supplementation frequency (FREQ) on milk protein yield. SEM = 8.6. (*FREQ* = 0.13). (*FREQ x d* = 0.97).

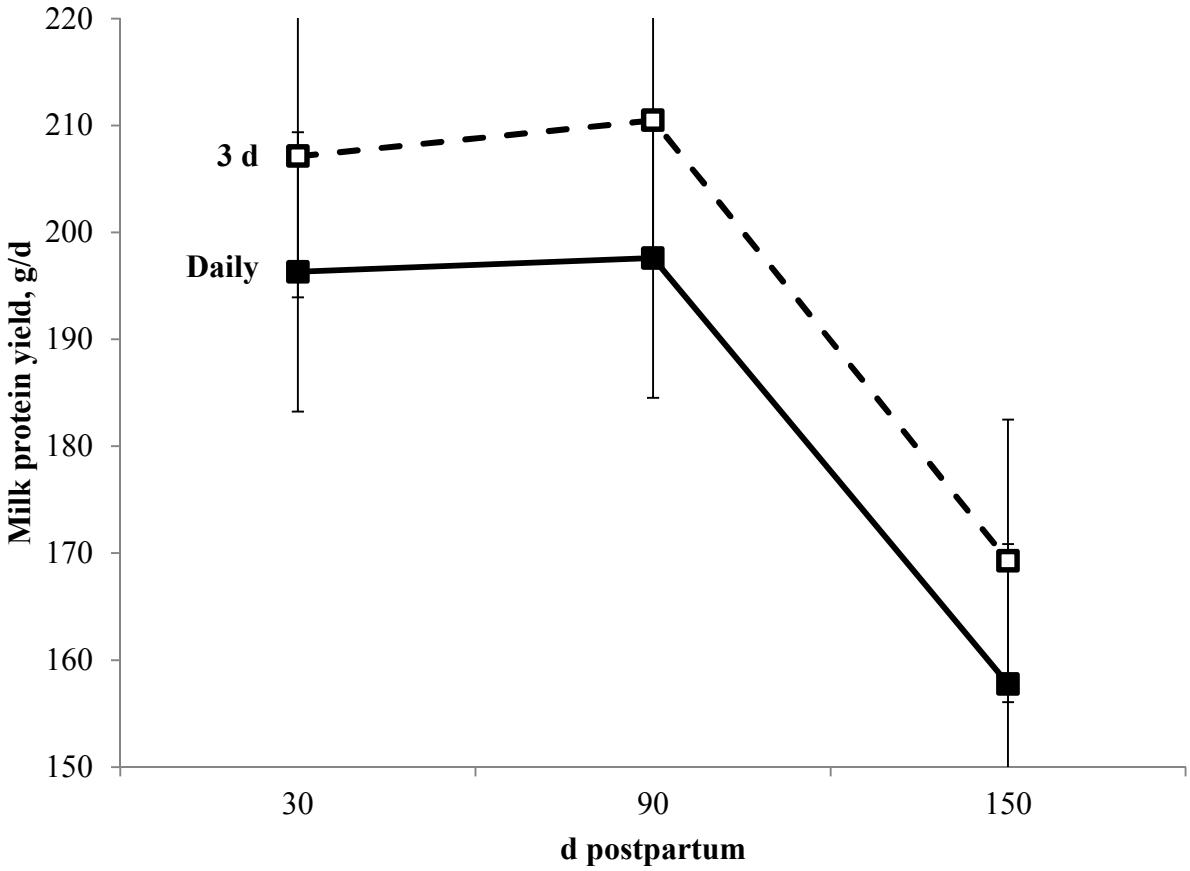


Figure 20. Effect of supplemental enzymatically hydrolyzed yeast (EHY) on milk protein yield. SEM = -7.6. (*EHY* = 0.69). (*EHY x d* = 0.49).

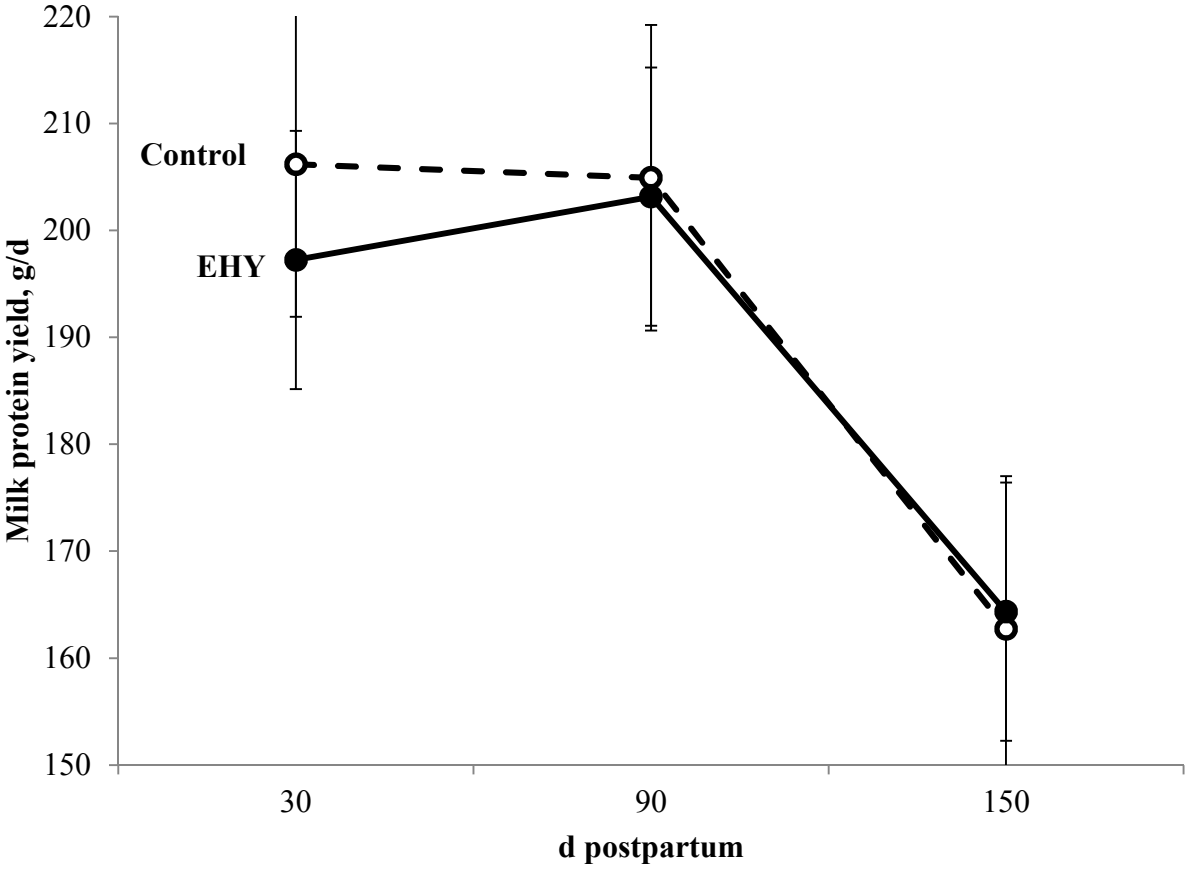


Figure 21. Effect of supplementation frequency (FREQ) on somatic cell count (SCC). SEM = 0.3. (*FREQ* = 0.40). (*FREQ x d* = 0.88).

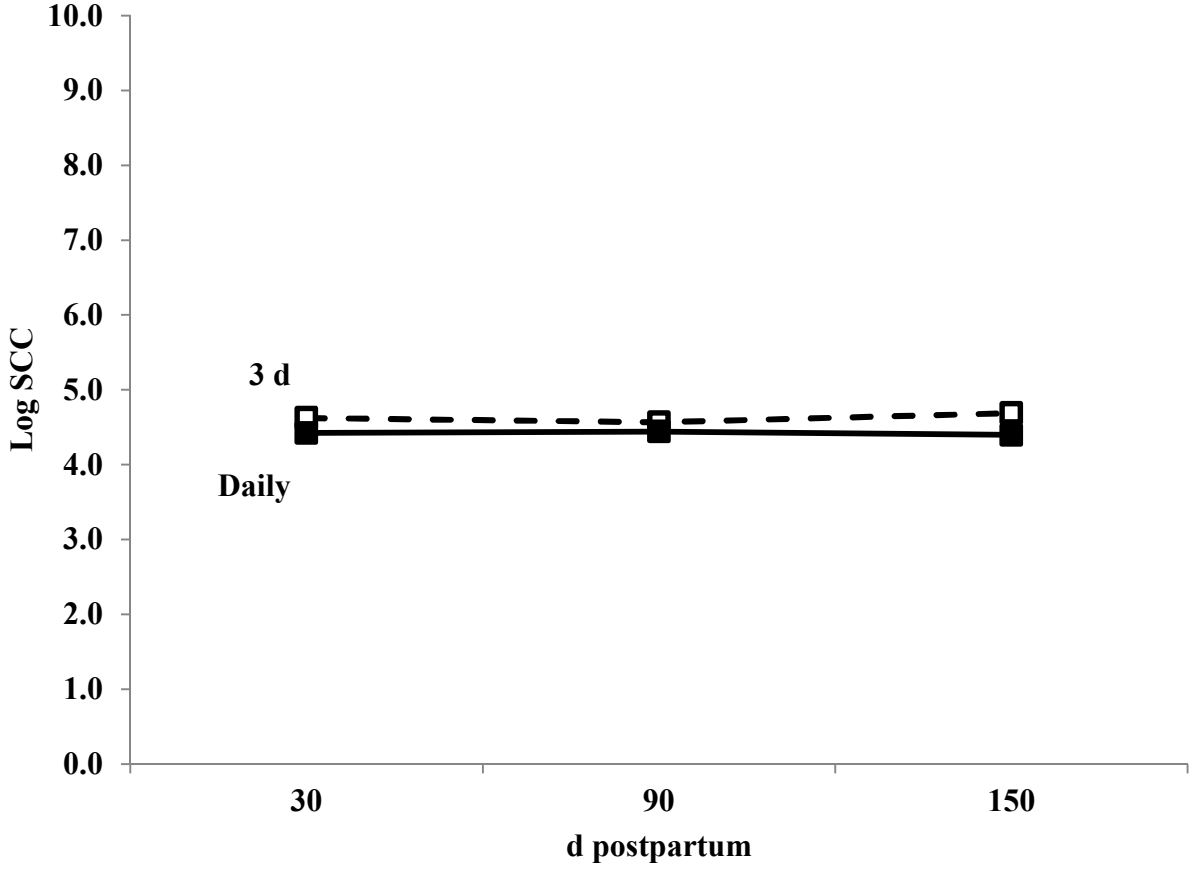
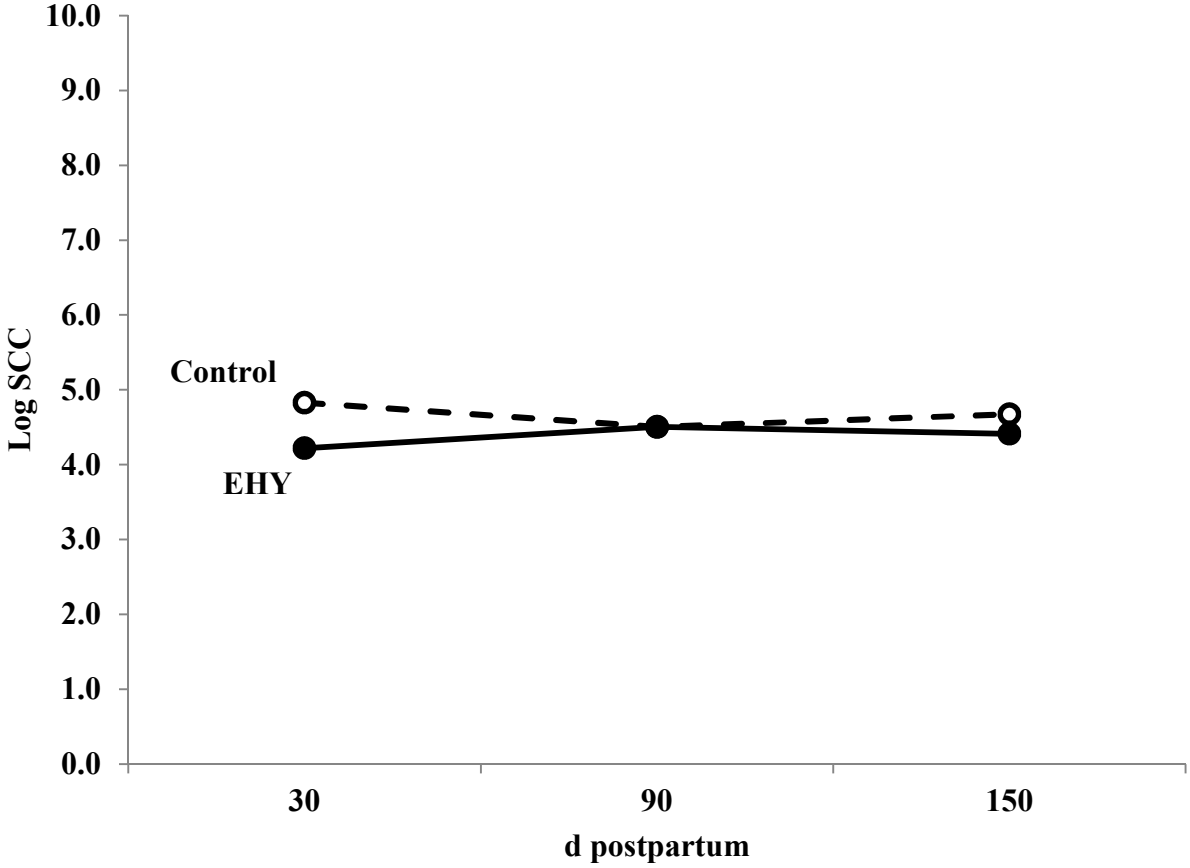


Figure 22. Effect of supplemental enzymatically hydrolyzed yeast (EHY) on somatic cell count (SCC). SEM = 0.2. (*EHY* = 0.24). (*EHY x d* = 0.17).



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