Effects of hydrolyzed yeast supplementation in calf starter on the immune response of neonatal calves experimentally-challenged with microbes

M. H. Kim¹, J. K. Seo¹, C. H. Yun¹, H. S. Kim², J. H. Kim², S. J. Kang², J. Y. Ko³ and Jong K. Ha¹,*

¹Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul 151-742, Republic of Korea

²Dairy Science Division, National Institute of Animal Science, Cheonan 330-801, Republic of Korea

³R&D center, Livestock Research Institute, National Agricultural Cooperative Federation, Anseong, Republic of Korea

*Corresponding Author: Jong K. Ha. Tel: +82-2-880-4809, Fax: +82-2-785-8710,
E-mail: Jongha@snu.ac.
Abstract

The effects of hydrolyzed yeast (HY) supplementation on growth performance, immune-physiological parameters, and health in neonatal calves with experimentally-induced microbial infection were investigated. Twelve Holstein calves were started in the experiment at 2 ± 1 d of age and were studied for 42 d. Three male and three female calves were randomly assigned to each of two dietary treatments, a control and an HY group. The calves in the HY group received control calf starter supplemented with 0.2% HY. All calves were given calf starter *ad libitum* for five weeks starting in week 1. Calves were also given whole milk according to a step-down milking protocol.

In order to induce microbial infection, all calves were challenged with *Hog cholera* and *Erysipelothrix insidiosa* live vaccines by intramuscular injection at three weeks old. Growth performance and feed intake were not affected by dietary treatment throughout the experimental period, except that the HY group had significantly higher ($P < 0.05$) milk intake than did the control group at three weeks of age. Calves in the HY group showed significantly better ($P < 0.05$) fecal and health scores at three weeks compared to those in the control group. After microbial infection, neutropenia, lymphophilia, and thrombocytopenia were observed in the control group, but calves in the HY group did not show significant changes of leukocytes. Feeding HY supplemented calf starter resulted in a higher ($P < 0.05$) relative amount of bacterial and viral-specific IgA than in the control group at five days post microbial infection (DPMI). The average concentration of serum haptoglobin in the HY group was significantly higher ($P < 0.05$) at three DPMI than that of control group. Although the percentage of CD4$^+$ T cells was significantly ($P < 0.05$) higher in the HY group than in the control group at -2 DPMI, significant differences between groups after microbial infection was not observed during the experimental period. These results suggest that 0.2% HY supplementation in calf starter can improve the health status and immune-related serum protein production and affect blood cell composition in neonatal calves after microbial infection.

Key words: neonatal calf, yeast-based feed ingredient, immune

Abbreviation Key: APP = acute phase protein, ANF = anti-nutritional factor, NE = neutrophil, LY = lymphocyte, DPMI = days-post microbial infection, Lf = lactoferrin, Hp = haptoglobin, SAA = serum amyloid A, LBP = lipopolysaccharide binding protein, HY = hydrolyzed yeast

Introduction

Neonatal calves are highly susceptible to a variety of diseases, both because of the immaturity of their immune systems and because a variety of stresses can attenuate immune activity during the early phase of calf life (Hickey et al., 2003; Jonathan et al., 2006). Therefore the administration of an effective dietary immune enhancer or stress reducer may be beneficial for early development of the immune system, strengthening of immune competence, and prevention of immune attenuation caused by various
stresses in neonatal calves. Although the efficacy of administration of yeast 
*Saccharomyces cerevisiae* culture on growth performance and production traits has 
been studied for mature ruminants (Seymour et al., 1995; Kumar et al., 1997; 
Lesmeister et al., 2004; Daniele et al., 2009; Kim et al., 2009), studies on the effects of 
yeast culture on growth performance and blood parameters in neonatal calves are not 
readily available in the literature. Hydrolyzed yeast (HY) contains cell wall components 
and extracts of cell constituents which possess various biological functions. Yeast cell 
wall products such as mannoproteins and β-glucan may improve performance and 
overall health by preventing pathogenic bacteria from binding to intestinal epithelial 
cells or by modulating immune function (Spring et al., 2004; Davis et al., 2004; Wang et 
al., 2009). In addition, HY contains a high amount of soluble, bioactive particles which 
modulate the immune system (Jensen et al., 2007). Young calves are typically 
challenged by a variety of microbial infections, but studies on the effects of yeast-based 
products on immune-related serum proteins in neonatal calves with microbial infections 
are not readily available in the literature.

Thus, the objective of this study was to evaluate the effects of yeast supplementation 
on growth performance, health and immune-physiological parameters in calves 
experimentally challenged with microbes. We used HY in our study and hypothesized 
HY in the calf diet of neonatal calves would improve immunocompetence against 
microbial infection through facilitation of immune-related serum protein production and 
modulation of leukocytes composition.

**Materials and methods**

**Animals and diets**

All experiments were carried out in the Dairy Science Division at the National 
Institute of Animal Science, South Korea. All experimental procedures were reviewed 
and approved by the Ethics Committee on the Use of Animals in Research of the 
National Livestock Research Institute, South Korea. Holstein calves (n = 12; six male 
and six female, BW = 36.23 ± 3.01 kg) were separated from their mothers within 2 h of 
birth, weighed, and moved into individual pens (1.5 × 2.5 m; bedded with wood 
shavings), where they were fed colostrum in the amount of 10% of their body weight for 
the first three days. The pens had solid iron rod sides with openings in the front and 
rear to allow calves free access to calf starter, chopped mixed grass hay and water 
from a bowl drinker in each pen. Calf starter and mixed grass hay were given from the 
first and fourth week of age, respectively. All calves were fed whole milk using mobile 
plastic bottles (2 L capacity) fitted with soft rubber nipples, using the step-down milking 
method (Khan et al., 2007). The amount of milk provided was 20% of body weight until 
28 days of age, and then at between 29 and 30 days old, the amount of milk was 
gradually reduced to 10% of body weight, which was maintained until the end of the 
experiment. Calves were fed calf starter supplemented with no HY (control) or 0.2% HY 
(Progut, Suomen Rehu Co. Ltd., Esbo, Finland) from the first week after birth. Both
starter diets were formulated to contain identical nutrients (CP=17.50, FAT=2.57, ASH=6.44, C-TDN=71.43) except that the treatment diet contained 0.2% HY.

**Feed intake**

Intakes of starter, milk and forage were recorded from two to five weeks of age. Overall average body weight (BW) gain, total dry matter intake (DMI: milk solids, calf starter and forage), and feed efficiency (kg of BW gain/kg of total DMI) were also calculated.

**Fecal and health scoring**

The scoring system used to monitor the overall health conditions of the calves was as follows: For respiratory scoring, the following scale was used: normal, 1; slight cough, 2; moderate cough, 3; moderate to severe cough, 4; severe and chronic cough, 5. Fecal scoring as determined by fecal fluidity, consistency and odor was conducted daily (0800 h) using the procedure of Larson et al. (1977). Fecal scoring was performed as follows: for fecal fluidity, normal, 1; soft, 2; runny, 3; watery, 4; for fecal consistency, normal, 1; foamy, 2; mucous-like, 3; sticky, 4; constipated, 5; for fecal odor, normal, 1; slightly offensive, 2; highly offensive, 3. When the fecal score exceeded 3 (average of fluidity, consistency and odor for two consecutive days) or when a calf exhibited other signs of disease (fever or cough), antibiotic therapy (sulfadimethoxine sodium, 55 mg/kg of BW daily; Green Cross Veterinary Products Co. Ltd., Yongin, South Korea) was initiated, and the therapy was continued until all visual signs of disease disappeared or for a maximum of 5 d. Scours were also treated with electrolyte therapy (Eltradd, 3 g/L in drinking water; Bayer Animal Health Co., Suwon, South Korea). Health score was determined as the average of respiratory score, fecal score and days of antibiotic and electrolyte therapy.

**Experimental infection and blood sampling**

In order to investigate the immune response after microbial infection, all calves were challenged with 1 ml porcine *Hog cholera* and *Erysipelothrix insidiosa* live vaccine (Green Cross Veterinary Products Co. Ltd., Yongin, South Korea) by intramuscular injection at three weeks of age (0 DPMI: days post–microbial infection). Blood samples were collected from jugular veins at 19 (-2 DPMI), 26 (5 DPMI), 35 (14 DPMI), and 40 (19 DPMI) days of age. For the determination of lactoferrin and haptoglobin concentrations, additional blood samples (10 ml) were collected into evacuated tubes coated with the anti-coagulant lithium-heparin (BD vacutainer, BD-Plymouth, UK) at 22 (1 DPMI) and 24 (3 DPMI) days of age.

**Hematology**

Plasma was harvested from anti-coagulated blood after centrifugation at 1,600 × g at 4°C for 15 min and stored at -80°C until subsequent assays were conducted.
Neutrophil (NE), lymphocyte (LY), platelet, monocyte and leukocyte populations in whole blood were measured on an automatic analyzer (Hemavet 850. Drew Scientific, Portsmouth, RI, USA).

Enzyme-linked immunosorbent assay (ELISA)

The amount of lactoferrin in plasma was determined using commercial ELISA kits based on lactoferrin binding capacity (Bethyl Laboratory, Montgomery, TX, USA). In brief, 96-well immunoplates (Nalgene Nunc International, Rochester, NY, USA) were coated with bovine lactoferrin capture antibody and incubated for one h. The plates were then washed with washing buffer (0.05% Tween 20 in PBS) three times and blocked with blocking buffer (1% bovine serum albumin (BSA) in PBS) for 30 min. After washing, the plates were incubated with bovine serum and respective standard proteins for one h at room temperature. The detection antibody conjugated with biotin was added to the plates after washing and incubated for one h. Specific binding was detected using streptavidin–HRP and TMB substrate (Sigma–Aldrich, St. Louis, MO, USA). The reaction was stopped with 2 N H$_2$SO$_4$. Absorbance was measured at 450 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA). Plasma haptoglobin (Life Diagnostics, Inc. West Chester, PA, USA) and total serum immunoglobulin concentrations (Bethyl Laboratory) were measured using ELISA assay kits according to the manufacturer’s instructions.

Levels of antigen-specific Ig in the serum samples were determined by indirect ELISA. *Hog cholera* and *Erysipelothrix insidiosa* live vaccine dissolved in PBS (1 ml) was centrifuged at 13,000 x g for 1 min. The supernatant was harvested and transferred into new tubes for virus collection, and the pellet was dissolved in PBS (1 ml) for the isolation of bacteria. Bacteria were sonicated (Vibracell, Danbury, CT, USA) and centrifuged at 50,000 x g for two hr at 4°C, and protein concentration in the supernatant was determined using a Bradford assay (Bradford, 1976). Each well of a microtiter plate was coated with bacterial antigen (10 μg/ml) or viral antigen (1:100 dilution). Antigen-specific IgG and IgA concentrations were measured using a bovine IgG and IgA ELISA assay kit (Bethyl Laboratory) according to the manufacturer’s recommendations. In brief, the wells of 96-well immunoplates (Nalgene Nunc International, Rochester, NY, USA) were coated with bacterial or viral antigens and incubated overnight at 4°C. The plates were then washed with washing buffer (0.05% Tween 20 in PBS) three times and blocked with blocking buffer (1% BSA in PBS) for two h. After washing, the plates were incubated with diluted bovine serum for three h at room temperature. Anti-bovine IgG (or IgA) HRP-conjugated antibody was added to the plates after washing, and the plates were incubated for two h. Specific binding was detected using streptavidin–HRP and TMB substrate (Sigma–Aldrich, St. Louis, MO, USA). The reaction was stopped with 2 N H$_2$SO$_4$. The absorbance was measured at 450 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).
Quantification of CD4* and CD8* T Cells

A single-color flow cytometric analysis was performed as follows: 5 x 10^5 cells were harvested and stained with CD4-APC or CD8-APC (VMRD, Pullman, WA, USA). After staining for 30 min at 37°C, cells were washed three times with PBS, and differences in the expressions of cell surface molecules were detected using a FACScanto flow cytometer (BD Bioscience, San Jose, CA, USA). All data files were further analyzed with the FACSDiva™ software program (BD Bioscience, San Jose, CA, USA).

Statistical Analysis

Data were analyzed using the ANOVA procedure. (SAS Institute Inc., Cary, NC) (2002). Differences among means were tested using the least significant difference (LSD) procedure of SAS (1996). Effects were considered significant at \( P < 0.05 \).

Results

Growth performance, feed intake and health

Body weight in the calves fed two different calf starters was similar throughout the experimental period (Table 1). Both feed intake and efficiency were also similar between groups, except that milk intake was lower in calves fed control calf starter compared to that of the HY group at three weeks of age (31.73 L vs. 36.00 L; \( P < 0.05 \)) (Table 1). This difference was mainly due to milk restriction for some calves in the control group due to diarrhea. The days of feed restriction were fewer for the calves in the HY calf starter group compared to those in the control group (data not shown). As shown in Table 2, calves fed HY calf starter showed significantly lower (\( P < 0.05 \)) fecal scores at three weeks than did the control calves. Calves on HY starter also showed lower (\( P < 0.05 \)) health scores, due to lower respiratory scores, at the same time.

Hematology

Dietary treatment influenced both leukocyte percentage and platelet levels after microbial infection (Table 3). Calves fed control calf starter showed decreased NE (%) and increased LY (%) (\( P < 0.05 \)) at 14 and 19 DPMI compared to those at -2 DPMI. Calves in the control group also showed decreased NE:LY (\( P < 0.05 \)) at 5, 14, and 19 DPMI compared to those at -2 DPMI, due to higher LY and lower NE. However, calves on HY starter did not exhibit this leukocyte population change. Platelet concentration decreased (\( P < 0.05 \)) in the control group at 5 DPMI, returning to the initial level thereafter. This decrease was not observed in the HY group. The concentration of leukocytes at 19 DPMI was lower (\( P < 0.05 \)) in both groups compared to the concentration at -2 DPMI.

Total and antigen-specific immunoglobulin production

Total serum IgG concentration in calves fed control calf starter significantly increased (\( P < 0.05 \)) at 14 and 19 DPMI compared to the concentration at -2 DPMI, while no such
increase was observed in the HY group (Table 4). Microbial infection did not influence
the level of bacteria (Erysipelothrix insidioussa)-specific or virus (Hog cholera)-specific
IgG in either group (Figures 1A and B). A gradual elevation of total serum IgA
concentration in calves fed both control and HY calf starter was observed (Table 4);
however, there was no significant difference between groups. There was a significant
difference (P < 0.05) in antigen specific IgA between calves fed different starters
(Figures 1C and D). Antigen-specific IgA concentrations increased after microbial
infection, with a more noticeable increase in the HY group. Calves fed HY-
supplemented calf starter showed drastic elevation at an earlier time compared to the
control group, although differences in bacteria- and virus-specific IgA concentrations
between the groups were only significant at 5 DPMI. Bacteria-specific IgA
concentrations reached their highest levels at 5 DPMI and 14 DPMI in the HY and
control group, respectively. Virus-specific IgA concentrations reached their highest
levels at 5 DPMI and 19 DPMI in the HY and control group, respectively.

Lactoferrin and haptoglobin concentrations
Microbial infection decreased serum lactoferrin concentrations in both control and HY
calves, with a more drastic decrease in the control group (Table 5). The lactoferrin
levels of the control group dropped (P < 0.05) from 534.95 ± 93.0 ng/ml at resting stage
(-2 DPMI) to 201.94 ± 49.7 ng/ml at 1 DPMI. A similar reduction was observed for HY
calves, with a significant decrease (P < 0.05) at 3 and 14 DPMI compared to -2 DPMI.
Feeding HY calf starter resulted in a significant elevation of serum concentration of
haptoglobin (Hp) at 3 DPMI compared to -2 DPMI, without such a change in the control
group (Table 5).

Change in T cells
Calves fed HY calf starter had higher (P < 0.05) CD4+ T cell levels before microbial
infection (-2 DPMI) than did those in the control group. The percentage of CD4+ T cells
in the HY group tended to be reduced until 14 DPMI, followed by a slight increase.
However, microbial infection did not influence CD4+ T cell levels of calves in the control
group (Figure 2A). Significant differences in the CD4+ and CD8+ cell populations
between groups after microbial infection were not detected (Figures 2A and B). The
CD4+/CD8+ ratio also did not differ between groups during the experimental period. The
CD4+/CD8+ ratio in the HY group showed a reduction tendency until 14 DPMI.

Discussion
Growing concern over the use of antibiotics and other growth promoters in the
animal feed industry has resulted in global research efforts to find alternative microbial
feed additives. Brewer’s yeast or live yeast have been considered as possible
candidates. Yeast has been typically included in calf diets at levels between 0.001%
and 1.00%, with some positive effects on DM intake, rumen pH, and nutrient
digestibility (Callaway and Martin, 1997; Kummar et al., 1997; Dann et al., 2000;
Wagner et al., 1990; Quigley et al., 1992). Previous studies have explained the beneficial effects of yeast products as due to yeast cell components and fermented products such as organic acids, vitamins, and nucleotides. However, inconsistent results due to the level and the source of the yeast culture product also have been reported (Williams et al., 1991; Lesmeister et al., 2004). In the present study, inclusion of hydrolyzed yeast at 0.2% in calf starter did not have any influence on the growth performance of calves. This is probably because the amount of calf starter consumed by the calves during the early phase of growth was not enough to make an appreciable impact on growth. Therefore, growth of calves might depend more on intake of milk rather than that of calf starter. In addition, the supplementation level of 0.2% might not have been high enough to make a significant difference in growth performance.

Components of the yeast cell wall, such as oligosaccharides, β-glucan and mannan, or the many metabolites contained in yeast culture, might not only benefit local and systemic immune responses, but might also have antimicrobial activities against pathogens (Murphy et al., 2007; Jensen et al., 2008). Recent studies reported that yeast cell wall components interact with immune systems, stimulating macrophage activation (Selijelid et al., 1987; Djeraba and Quere, 2000). Previous studies also reported that supplementation with 2% yeast culture improved fecal scores, reduced days with watery feces, and reduced risk of health disorders in Holstein calves, and also that incorporating live yeast into the grain feed reduced the number of days the calves were afflicted with diarrhea (Galvão et al., 2005; Magalhães et al., 2008). Nevertheless, few studies on the efficacy of yeast or yeast products on health or immune activity in young calves are available (Seymour et al., 1995; Galvão et al., 2005; Magalhães et al., 2008). In the present study, at three weeks of age, calves fed HY calf starter showed lower fecal scores and health scores than did those calves fed an HY-absent diet, indicating that HY had positive effects on the prevention of diarrhea as well as on the improvement of general health conditions. Although it is not apparent from our study how HY improved the health condition of calves, it can be postulated that some yeast cell wall components such as mannoproteins, β-glucan, and oligosaccharides might have decreased the attachment and invasion of intestinal cells by pathogens, because pathogenic bacteria might be directed to bind to cell wall components (White et al., 2002). Attachment of a pathogen to the cell wall of *S. cerevisiae* and subsequent reduction in the attachment and invasion by the pathogen to intestinal cells has been proposed (Pérez-Sotelo et al., 2005).

In the present study, all calves were challenged with porcine live bacterial and viral vaccine by intramuscular injection at three weeks age in order to evaluate the effect of HY supplementation on the systemic immune response against microbial infection. Microbial infection increased total serum IgA and antigen-specific IgA in both groups. However, microbial infection did not influence total or antigen-specific IgG. These results suggest that porcine live vaccine preferentially induced the IgA immune response.
It is notable that bacterial- and viral-specific IgA levels in the calves fed with HY calf starter were significantly higher than those of the control group at 5 DPMI. These results suggest that calves on HY calf starter produced antigen-specific IgA more efficiently against microbial infection compared to those on control calf starter without HY. A direct explanation on these effects is not possible from data obtained in the present study. However, it is possible that β-glucan, nucleotides, or small peptides in HY contributed to the production of serum IgA. Many studies have reported the relationship between small-sized peptide ingestion and Ig concentration in blood. Wang and colleagues (2003) demonstrated that adding 3 g of small peptides per kg body weight to the basal diets of piglets increased the concentration of immunoglobulin, and Feng et al. (2007) showed that fermented soybean meal (SBM) having small-sized peptides increased the level of IgA and IgM, but not IgG, in broiler chickens. In chickens, IgG concentrations were greater in animals receiving mannan-oligosaccharide (MOS) compared with those of control animals (Savage et al., 1996). In addition, it has been reported that yeast contains a variety of bioactive components, such as MOS, which might have positive effects on IgG concentrations and lymphocyte transformation (Savage et al., 1996; Davis et al., 2002; White et al., 2002).

Acute phase proteins (APPs) provide enhanced protection against microorganisms and modify inflammatory responses through cell trafficking and mediator release (Suffrendini et al., 1999). Haptoglobin (Hp) is considered to be a representative APP in cattle. Although there was large variation among individual calves, the average concentration of Hp increased significantly in the HY group in response to microbial infection, while significant change of Hp in the control group was not observed. Emmanuel et al. (2007) proposed a mode of action for probiotics in the elevation of APP. It is of note that the production of efficient Hp after microbial infection is thought to provide beneficial effects on immune responses against incoming pathogens through modulation of inflammatory activities.

Lactoferrin (Lf), an iron-binding glycoprotein, is known to exert bactericidal activity as well as immunoregulatory functions upon microbial challenge by modulating IL-1, IL-2 and tumor necrosis factor (TNF)-α (Caccavo et al., 2002). Although the effect of lactoferrin as an immune enhancer has been well studied, lactoferrin levels in the blood circulation and its relevance during microbial infection has not been assessed in cattle. In the present study, a significant reduction of Lf levels in the control group and a non-significant reduction in the HY group were observed. Reduction of Lf levels in blood circulation might be explained by Lf migrating into the local site of infection to participate in an immune response, resulting in the reduction of circulating Lf. Further studies on the role of circulating Lf after microbial infection are necessary.

Many studies have reported that yeast or yeast products influence blood cell composition in weanling pigs or lipopolysaccharide (LPS) -challenged piglets (Davis et al., 2004; Shen et al., 2009). Davis et al. (2004) found a tendency toward a lower level of neutrophils and a greater proportion of lymphocytes in blood of mannan-fed
weanling pigs. In addition, Nonnecke et al. (2003) reported that total numbers of blood leukocytes and the composition of the mononuclear leukocyte population from neonatal calves were affected by level of energy and protein intake. To obtain more insight into the effects of yeast products on blood cell composition in an experimental infection model using neonatal calves, we conducted blood cell composition analysis. A previous study reported that cattle infected with *M. bovis* showed systemic responses, including leukopenia, lymphopenia, neutropenia and thrombocytopenia, during the first few days of the infection, and it has also been reported that leukopenia occurs early in bacterial infections in ruminants (Kauf et al., 2007; Kahn et al., 2008). In the present study, different tendencies for changes in blood cell composition were observed between groups after microbial infection. Neutropenia, lymphophilia, thrombocytopenia and reduction of NE:LY were observed only in the control group, while these symptoms were absent in the HY group. This suggests that HY improved the prognosis after microbial infection, as suggested by Kahn et al. (2008).

Davis et al. (2004) reported a lower CD3⁺CD4⁺:CD3⁺CD8⁺ T lymphocyte ratio in jejunal lamina propria tissue of mannan-fed pigs compared with pigs fed the basal diet. In our study, significant differences between groups after microbial infection was not observed except for a lower CD4⁺ T cell percentage in the control group. Further studies on change of cytokines level and lymphocyte population after microbial infection are necessary in order to investigate the mechanism of the effects of yeast products on immune response.

**Conclusions**

Our data suggest that supplementation with hydrolyzed yeast (HY) at the 0.2% level in calf starter improved general health conditions during the neonatal period. Hydrolyzed yeast also had positive hematological prognostic signs and promoted the production of immune-related serum proteins, particularly IgA and haptoglobin, after microbial infection. This may be considered to be an indication of better immunocompetence against microbial infection. Further studies are necessary in order to identify the exact components of hydrolyzed yeast that have a positive effect on immune response in neonatal calves.

**Acknowledgement**

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References


Table 1. Body weight and intake of calf starter, milk, forage, total DMI and feed efficiency in calves fed calf starters with or without hydrolyzed yeast (HY)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>HY</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>38.17a</td>
<td>36.67a</td>
<td>1.01</td>
</tr>
<tr>
<td>7 days</td>
<td>40.83a</td>
<td>38.25a</td>
<td>1.23</td>
</tr>
<tr>
<td>14 days</td>
<td>45.17a</td>
<td>41.67a</td>
<td>1.45</td>
</tr>
<tr>
<td>28 days</td>
<td>50.67a</td>
<td>47.67a</td>
<td>1.33</td>
</tr>
<tr>
<td>42 days</td>
<td>56.75a</td>
<td>54.08a</td>
<td>1.30</td>
</tr>
<tr>
<td><strong>Calf starter intake</strong> (g/week)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 wk</td>
<td>172a</td>
<td>342a</td>
<td>19.3</td>
</tr>
<tr>
<td>3 wk</td>
<td>539a</td>
<td>510b</td>
<td>28.0</td>
</tr>
<tr>
<td>4 wk</td>
<td>693a</td>
<td>953a</td>
<td>35.8</td>
</tr>
<tr>
<td>5 wk</td>
<td>1358a</td>
<td>1505a</td>
<td>102.9</td>
</tr>
<tr>
<td><strong>Milk intake (L/Week)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2 wk</td>
<td>36.90a</td>
<td>34.80a</td>
<td>1.71</td>
</tr>
<tr>
<td>3 wk</td>
<td>31.73b</td>
<td>36.00a</td>
<td>1.63</td>
</tr>
<tr>
<td>4 wk</td>
<td>27.90a</td>
<td>27.00a</td>
<td>1.01</td>
</tr>
<tr>
<td>5 wk</td>
<td>20.40a</td>
<td>20.00a</td>
<td>0.92</td>
</tr>
<tr>
<td><strong>Total forage intake (g)</strong></td>
<td>590.22a</td>
<td>581.12a</td>
<td>33.8</td>
</tr>
<tr>
<td>Total DMI (kg)</td>
<td>32.12a</td>
<td>31.30a</td>
<td>1.92</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>0.57a</td>
<td>0.55a</td>
<td>-</td>
</tr>
</tbody>
</table>

1. Total DMI = milk solid, starter and forage DMI during experimental period.
2. Feed efficiency = kg of BW gain/kg of total DMI.
3. Means with different letters differ significantly between groups (P < 0.05).
Table 2. Average fecal and health scores in calves fed calf starter with or without hydrolyzed yeast (HY) (mean ± SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>HY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fecal score</strong>¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 wk</td>
<td>2.37 ± 0.5ᵃ</td>
<td>3.82 ± 2.3ᵃ</td>
</tr>
<tr>
<td>3 wk</td>
<td>5.98 ± 2.1ᵃ</td>
<td>1.35 ± 0.6ᵇ</td>
</tr>
<tr>
<td>4 wk</td>
<td>1.25 ± 0.4ᵇ</td>
<td>2.01 ± 1.3ᵃ</td>
</tr>
<tr>
<td>5 wk</td>
<td>2.63 ± 1.1ᵃ</td>
<td>3.41 ± 1.8ᵃ</td>
</tr>
<tr>
<td><strong>Health score</strong>²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 wk</td>
<td>3.52 ± 1.0ᵃ</td>
<td>4.01 ± 1.4ᵃ</td>
</tr>
<tr>
<td>3 wk</td>
<td>7.01 ± 3.0ᵃ</td>
<td>4.12 ± 1.8ᵇ</td>
</tr>
<tr>
<td>4 wk</td>
<td>3.29 ± 1.9ᵇ</td>
<td>3.56 ± 1.9ᵃ</td>
</tr>
<tr>
<td>5 wk</td>
<td>3.00 ± 1.6ᵃ</td>
<td>3.89 ± 2.0ᵃ</td>
</tr>
</tbody>
</table>

¹ Means with different letters differ significantly between groups in each week (P < 0.05).
² Fecal score = Average of fecal consistency, fluidity, and odor.
³ Health score = Average of fecal score, respiratory score, and days of therapy.
Table 3. Changes in leukocytes and platelet levels (mean ± SE) in calves fed calf starter with or without hydrolyzed yeast (HY)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Control</th>
<th>HY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neutrophil (%)</td>
<td></td>
</tr>
<tr>
<td>-2 DPMI</td>
<td>44.03 ± 3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.45 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>5 DPMI</td>
<td>38.70 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.35 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>14 DPMI</td>
<td>33.56 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.36 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>19 DPMI</td>
<td>32.29 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.40 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Lymphocyte (%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>-2 DPMI</td>
<td>48.63 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.58 ± 4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>5 DPMI</td>
<td>54.09 ± 2.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>53.01 ± 4.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>14 DPMI</td>
<td>59.89 ± 4.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.30 ± 4.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>19 DPMI</td>
<td>61.39 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.15 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Neutrophil:Lymphocyte</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>-2 DPMI</td>
<td>0.95 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.67 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>5 DPMI</td>
<td>0.71 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.78 ± 0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
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</tr>
<tr>
<td>14 DPMI</td>
<td>0.56 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.74 ± 0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>19 DPMI</td>
<td>0.53 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.68 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Leukocytes (10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td></td>
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<td></td>
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<tr>
<td>-2 DPMI</td>
<td>9.80 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.84 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>5 DPMI</td>
<td>8.92 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.56 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>14 DPMI</td>
<td>9.03 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.00 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>19 DPMI</td>
<td>7.86 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.65 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Platelets (10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td></td>
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<td></td>
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<tr>
<td>-2 DPMI</td>
<td>476.50 ± 22.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>569.83 ± 41.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>5 DPMI</td>
<td>334.75 ± 47.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>529.75 ± 32.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>14 DPMI</td>
<td>401.17 ± 31.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>527.52 ± 20.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>19 DPMI</td>
<td>417.33 ± 21.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>486.81 ± 21.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> DPMI = Days post microbial infection (0 DPMI = day of microbial infection, 21 days old).

<sup>ab</sup> Means with different letters differ significantly (P < 0.05).
Table 4. Changes in total serum antibodies (mean ± SE) in calves fed calf starter with or without hydrolyzed yeast (HY)

<table>
<thead>
<tr>
<th></th>
<th>Serum IgG (mg/ml)</th>
<th>Serum IgA (μg/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>HY</td>
</tr>
<tr>
<td>-2 DPMI</td>
<td>12.65 ± 2.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.25 ± 2.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 DPMI</td>
<td>17.16 ± 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.23 ± 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>14 DPMI</td>
<td>25.72 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.92 ± 3.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>19 DPMI</td>
<td>25.64 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.24 ± 4.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup><sup>b</sup>Means with different letters differ significantly (P < 0.05).
Table 5. Changes in lactoferrin and haptoglobin (mean ± SE) levels in serum obtained from calves fed calf starter with or without hydrolyzed yeast (HY)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Control</th>
<th>HY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Lactoferrin (ng/ml)</td>
<td></td>
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<td></td>
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<tr>
<td>-2 DPMI</td>
<td>534.95 ± 93.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>431.85 ± 41.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>1 DPMI</td>
<td>201.94 ± 49.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>368.57 ± 68.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3 DPMI</td>
<td>209.03 ± 36.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>327.07 ± 38.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>5 DPMI</td>
<td>200.98 ± 46.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>393.97 ± 78.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>14 DPMI</td>
<td>350.12 ± 35.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>320.23 ± 45.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Haptoglobin (g/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2 DPMI</td>
<td>14.78 ± 5.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.98 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>1 DPMI</td>
<td>14.79 ± 4.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.48 ± 30.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3 DPMI</td>
<td>37.32 ± 10.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>169.24 ± 51.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>5 DPMI</td>
<td>8.10 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.64 ± 12.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>14 DPMI</td>
<td>10.24 ± 1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.84 ± 2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup><sup>ab</sup></sup>Means with different letters differ significantly (P < 0.05).
Figure 1. Change in antigen-specific antibodies in serum obtained from calves fed control (○) or HY (■) calf starter during the experimental period. Relative concentrations of bacteria-specific IgG (A), virus-specific IgG (B), bacteria-specific IgA (C), and virus-specific IgA (D)

Means with different letters differ significantly between groups (P < 0.05).
Figure 2. Changes in the phenotypic composition of the serum from calves fed with control (○) or HY (■) calf starter during the experimental period. Changes in CD4⁺ cell population (A), CD8⁺ cell population (B), CD4⁺/CD8⁺ ratio (C)

Means with different letters differ significantly between groups ($P < 0.05$).