CHARACTERISTICS OF LIVE AND KILLED BREWER’S YEAST SLURRIES AND INTOXICATION BY INTRARUMINAL ADMINISTRATION TO CATTLE

C. L. Bruning and M. T. Yokoyama
Michigan State University
East Lansing 48824--1225

ABSTRACT

The physical and nutritional characteristics of live and killed brewer’s yeast slurries and the possible toxicity of intraruminal administration of loading doses of these by-products were evaluated. Dry-matter (DM) percentages of live brewer’s yeast slurry ranged from 10.5 to 29.0, and DM percentages of killed brewer’s yeast slurry ranged from 14.6 to 18.5. Total crude protein (N × 6.25) percentages of live and killed yeast slurries (DM basis) were 44.1 and 43.1; ethanol percentages (wet basis) were 6.96 and 1.84, respectively. Phase contrast photomicrographs showed distinct differences in morphology between live and killed yeast cells. Intraruminal administration of loading doses (0, 2.3, 4.5 and 6.8 kg) of live and killed brewer’s yeast slurries to bull calves induced clinical intoxication at the 4.5-kg and 6.9-kg dosages of live brewer’s yeast slurry. No toxicity was induced either with the killed brewer’s yeast or with the 0-kg or 2.3-kg dose levels of live brewer’s yeast slurry. The clinical signs and plasma ethanol concentrations suggested ethanol intoxication. Ruminal NH₃ concentrations increased to over 70 mg/dl with the 4.5-kg and 6.9-kg dosages of live brewer’s yeast slurry, but they did not exceed 35 mg/dl with an equivalent dosage of killed brewer’s yeast slurry.

(Key Words: Brewer’s Yeast, Ethanol, Ammonia, Cattle.)

Introduction

Brewer’s yeast (Saccharomyces cerevisiae) is generally accepted as an alternative by-product feed for livestock because of its high nutritive value and relatively low toxicity (Carter and Phillips, 1944; Bhattacharjee, 1970). Limitations on the use of yeast, when supplemented in the diet of nonruminants, have been its high nucleic acid content, unpalatability and bulkiness. Small amounts are generally tolerated in the diet, but consumption of large quantities has resulted in digestive disturbances (Carter and Phillips, 1944). The use of this eukaryotic organism is still limited to small amounts in cattle diets as a source of vitamins and unidentified growth factors for stimulating intake and ruminal digestion (Beeson and Perry, 1952; LeGendre et al., 1955; Wiedmeier and Arambel, 1985) or as a microbial adjuvant for reducing stress (Phillips and Von Tungeln, 1984). Studies also have examined yeast for its potential as a protein supplement for cattle because of its high crude protein content. Brewer’s yeast slurry fed to dairy heifers either ad libitum, mixed or top-dressed on a corn silage ration resulted in no apparent digestive problems (Grieve, 1979). When supplemented at either 6% or 12% of dry matter (DM) in a complete ration for lactating cows, brewer’s yeast slurry did not affect milk yield, composition or quality (Steckley et al., 1979). Despite these promising results, there have been a few undocumented accounts of digestive disturbances in cattle fed live brewer's yeast slurry. Whether brewer’s yeast slurry can be safely fed live, or whether it must be killed before feeding, is not fully known.

The present study was conducted to examine physical and compositional characteristics of live and killed brewer’s yeast slurries and to determine the toxicity of these by-products dosed intraruminally in cattle.
Experimental Procedure

Source of Brewer's Yeast. Live and killed brewer's yeast slurries were obtained from Stroh Brewery Co., Inc., Detroit, MI. Live brewer's yeast was recovered from fermentation vats by centrifugation after it was allowed to settle for 2 h. The recovered slurry was cooled for an additional 2 h in a refrigerated room (4 C). Killed brewer's yeast was prepared at the brewery by sparging steam through the recovered slurry for at least 4 h to maintain a temperature of 71 C, followed by cooling the slurry to about 38 C to 43 C for handling. Both slurries were transported in 208-liter barrels and were stored either at 21 C or 4 C in closed containers. Cell viability was determined on well stirred samples of the slurry, using the methylene blue staining method (Borzani and Vairo, 1958). Enumeration was performed by direct microscopic counts of serially diluted samples using a Petroff-Hauser counting chamber (Koch, 1981). The percentage of live yeast cells in the slurry was calculated as the ratio of the number of cells stained by methylene blue subtracted from the total number of cells  100.

Analytical Procedures. Dry-matter percentage was determined gravimetrically by drying 100-g aliquots of well stirred slurries at 55 C for 48 h in a forced-air oven. Aliquots of each slurry were centrifuged at 20,000 x g for 30 min. The supernatant and pellet fractions were frozen at -20 C until they were analyzed for ethanol and crude protein. Crude protein (N × 6.25) contents of the dried yeast cells and slurry supernatant were determined as total Kjeldahl-N using a Technicon Autoanalyzer II (AOAC, 1980). Ethanol concentration in the slurry supernatant was determined by gas-liquid chromatography, using a Varian Aerograph Chromatograph (Model 1800). Oven temperature was isothermal (70 C) and gas flow (healing) was 25 ml/min. N-butanol (0.5%) was used as internal standard (Allison et al., 1964).

Resistance to mold growth was determined by placing 10-g aliquots of the live and killed yeast slurries into sterile petri dishes, exposing the dishes to a barn environment for 30 min and examining them daily for mold growth over 12 d at room temperature (21 C).

Animals and Treatments. Three ruminally cannulated Hereford bull calves, averaging 227 kg in body weight were used for the intraruminal loading dose studies. The calves were individually stanchioned in metabolism stalls, adjusted to corn silage as the total diet (8.0% crude protein) and fed twice daily, with water and trace mineral supplement provided ad libitum. The treatments consisted of four dosages (0, 2.3, 4.5 and 6.8 kg) of either live or killed brewer's yeast slurry. To minimize any effects of either a decrease in cell viability or change in composition during storage at room temperature, live brewer's yeast slurry was administered sequentially in increasing dosages after the residual respiratory activity had abated (e.g., 3 d of storage). In the first study, all calves received tap water (6.8 kg) intraruminally as the 0 treatment during period I, followed by periods II, III and IV, during which all calves received single doses of 2.3, 4.5 kg and 6.8 kg of the live brewer's yeast slurry, respectively. There was a 7-d recovery period between each dosage; all dosing took 28 d to complete. In the second study, killed brewer's yeast slurry stored at room temperature was substituted for the live yeast and was administered sequentially in increasing dosages after the residual respiratory activity had abated. All calves were intraruminally dosed with the appropriate amount of the slurries via their rumen cannulae, after an overnight fast, and they were immediately fed their morning ration.

Rumen and Plasma Analyses. Rumen fluid (250 ml) was collected at zero time (just prior to dosing) and at 3-h intervals for 12 h post-dosing, strained through two layers of cheesecloth, acidified with 50% sulfuric acid to pH 2.0 and centrifuged at 20,000 x g for 30 min. Ruminal NH3 concentration was determined potentiometrically on centrifuged (20,000 x g for 30 min) ruminal fluid, using a selective ammonium ion electrode (Orion Model 95–10) and a Beckman (Model 4500) digital pH meter. Ammonium chloride was used to establish a standard curve (EPA, 1974).

5 Technicon Corp., Tarrytown, NY.
6 Orion Research Inc., Boston, MA.
7 Beckman Instruments, Palo Alto, CA.
Jugular blood (20 ml) was collected into heparinized vacutainers at zero time (prior to dosing) and at 3-h intervals for 12 h postdosing. Blood samples were centrifuged at 3,000 x g for 10 min, and the plasma was frozen at −20 C for ethanol analysis. Plasma ethanol concentration was determined by gas-liquid chromatography, using N-butanol (5%) as the internal standard, as described for the analysis of the slurry supernatant fluid. Plasma samples (1.0 ml) were deproteinized with 50% sulfosalicylic acid (1 ml) and centrifuged at 10,000 x g for 20 min; 2 µl analyzed directly.

Results

Characteristics of Brewer’s Yeast Slurries. Live brewer’s yeast stored at room temperature posed an initial handling problem because the slurry had high respiratory activity. However, this activity gradually abated after 2 d of storage. Concomitant with this decrease in respiratory activity was a rapid decrease in viability of cells (figure 1). During the first 2 d of storage, when respiratory activity of the slurry was high, viability of yeast cells was 64%. However, after 3 d and 4 d of storage, cell viability sharply declined to 30%, and after 5 d only about 10% of the yeast cells were determined to be alive in the slurry. When live brewer’s yeast slurry was stored at 4 C and 2 wk, cell viability remained relatively high at 55%, and respiratory activity was more effectively kept under control.

Dry matter of the live brewer’s yeast slurry ranged from 10.5% to 29.0% with different shipments from the brewery and averaged 21.4%. Some of this variation in DM was due to sampling the slurry while the respiratory activity was still very high. When the activity abated, DM ranged from 10.5% to 14.0%. In contrast, the DM of killed brewer’s yeast slurry was much more consistent. Different shipments from the brewery ranged from 14.6% to 18.5%, averaging 17.1%.

Separate analyses of the live and killed yeast cells and slurry supernatants indicated a difference of .5 percentage units in crude protein content between the live (38.9%) and killed (39.4%) yeast cells (DM basis). Soluble crude protein contents of the supernatants from the live and killed slurries were 5.2% and 3.7% (DM basis), respectively. Total crude protein (cells and supernatant fluid) of the live and killed yeast slurries was 44.1% and 43.1% (DM basis, respectively. Gas chromatographic analyses indicated that ethanol contents were 6.96% and 1.84% (wet basis) for the live and killed brewer’s yeast slurries, respectively.

Phase contrast photomicrographs of the live and killed brewer’s yeast cells showed a distinct difference in their morphological characteristics (figures 2a, 2b). Live brewer’s yeast showed a distinct refractory area surrounding each cell, which was a physical phenomenon of their viability, and cells appeared to be rounder, with more structural integrity. In contrast, heat-killed brewer’s yeast lacked this refractivity, and although their cell walls were intact, they appeared to be irregular in shape and more highly aggregated. When stained with methylene blue, the heat-killed cells turned dark blue. Live cells did not absorb the dye as intensely. Live brewer’s yeast slurry was more resistant to spoilage by molds than killed brewer’s yeast slurry, despite being higher in soluble crude protein content. No mold growth was detected in petri dish cultures of live brewer’s yeast slurry for up to 12 d after exposure to a barn environment. Luxuriant mold growth

Figure 1. Viability of live brewer’s yeast slurry with days of storage at room temperature (21 C). Viability was determined by the methylene blue staining method, with enumeration by direct microscopic count. Means and standard errors of three determinations at each point.
**Acute Intoxication.** When live brewer's yeast slurry with 16% viability and 10.5% DM content was intraruminally administered, acute, dose-dependent intoxication was induced in all calves within 1 h after dosing. The symptoms that were observed at dosages of 4.5 kg and 6.8 kg included reluctance to stand, lethargy, stupor, staggering walk and loss of appetite. With the 4.5-kg dose, all of the calves recovered from the symptoms within 6 h and resumed eating within 12 h after dosing. With the 6.8-kg dose, the symptoms were similar, but much more severe, and recovery was much slower. The calves were unable to stand, were extremely comatose and did not resume eating until 18 to 24 h after dosing. One calf did not fully recover from symptoms until 30 h postdosing. No acute intoxication was induced with either the 0-kg or 2.3-kg dose levels of live brewer's yeast slurry. In marked contrast to these effects, intraruminal administration of killed brewer's yeast slurry, with 14.6% DM induced no symptoms of acute intoxication in calves at any of the equivalent dose levels (0, 2.3, 4.5, and 6.8 kg).

**Plasma Ethanol Concentration.** Plasma ethanol concentrations increased after intraruminal administration of increasing dosages of live brewer's yeast slurry. Administration of 2.3 kg of live brewer's yeast slurry resulted in a peak plasma ethanol concentration of 7.1 mg/dl at 3 h, followed by a steady decline until 12 h postdosing (figure 3a). When 4.5 kg of live brewer's yeast was administered, plasma ethanol concentrations peaked at 6 h at a concentration of 21.2 mg/dl and then rapidly declined. With the 6.8-kg dosage, plasma ethanol concentrations rapidly increased and remained elevated above 20 mg/dl through 12 h postdosing. Plasma ethanol concentrations also increased after the intraruminal administration of increasing dosages of killed brewer's yeast slurry (figure 3b). However, plasma ethanol concentrations for the 2.3-kg, 4.5-kg, and 6.8-kg dosages all peaked at 3 h postdosing, at concentrations that were considerably lower than that of the lowest dosage of live brewer's yeast slurry. The highest peak plasma ethanol concentration observed with the killed brewer's yeast slurry was 4.2 mg/dl.

**Ruminal Ammonia Concentration.** Ruminal fluid NH₃ concentration markedly increased with the intraruminal administration of live brewer's yeast slurry (figure 4a). When 2.3 kg was dosed, ruminal NH₃ increased to 27 mg/dl at 3 h, then rapidly declined. However, when 4.5 kg and 6.8 kg of live brewer's yeast slurry was dosed, ruminal NH₃ concentrations peaked between 6 to 9 h postdosing at over 70 mg/dl and remained above 50 mg/dl until 12 h postdosing. Ruminal NH₃ concentrations also increased with the intraruminal administration of killed brewer's yeast slurry (figure 4b). Ruminal NH₃ concentrations for all dose levels peaked at 3 h postdosing; the highest peak concentration at 32 mg/dl was for the 6.8-kg dose levels.

**Discussion**

The acute intoxication induced by the intraruminal administration of loading doses of live brewer's yeast slurry was clearly due to the high ethanol content of the slurry. Symptoms presented immediately after dosing, and plasma...
ethanol concentrations were extremely high by 3 h postdosing. Acute intoxication, similar in clinical signs to those observed in this study, was previously induced in a 500-kg cow intraruminally dosed daily with about 380 ml of ethanol (Emery et al., 1959). Based on its ethanol content, the live brewer's yeast slurry used in this study contained about 140, 250 and 420 ml of ethanol in the 2.3-kg, 4.5-kg and 6.8-kg dosages, respectively. In contrast, the killed brewer's yeast slurry contained about 40, 80 and 120 ml of ethanol in the equivalent dosages. The calves were able to tolerate ethanol administered up to 140 ml, but 250 ml and 420 ml were apparently toxic. The high ruminal NH₃ concentrations observed with the 4.5-kg and 6.8-kg dosages of live brewer's yeast slurry may have had a role in exacerbating the ethanol intoxication. However, the rapid recovery of the calves when they received the...
4.5-kg dosage suggested that the high NH$_3$ concentrations were not the primary cause of intoxication. Ruminal NH$_3$ concentrations in excess of 100 mg/ml, with no apparent NH$_3$ intoxication, were reported when large amounts of preformed protein were fed to steers (Fenderson and Bergen, 1976). With proper slurry management, ethanol intoxication should not be a problem unless a large quantity is rapidly consumed by animals.

The high residual activity of live brewer’s yeast also might cause a bloat problem if the by-product is fed ad libitum. Calves did not bloat in this study, but the viability of the yeast cells was too low to assess this potential problem, and the activity had abated before administration. Very few live cells are present in slurry after 2 wk of storage (Steckley et al., 1979a), and frothy activity can be controlled by killing the cells with formic acid or formaldehyde.

Changes in the composition of live brewer’s yeast slurry occur with temperature and storage time. A decrease in DM and true protein content, NH$_3$ content and N solubility occur with 14 d of storage at 21 C or higher (Steckley et al., 1979a). Except for a slightly higher soluble crude protein content in the supernatant, which was possibly caused by some autolysis during storage, live brewer’s yeast slurry was similar to killed brewer’s yeast slurry in total crude protein content. However, live brewer’s yeast slurry contained almost four times more ethanol than killed brewer’s yeast slurry, presumably caused by residual fermentation and by substantial loss of ethanol during the heat processing to kill cells. This higher ethanol content may be responsible for the higher resistance of live brewer’s yeast slurry to spoilage by molds.

The high ruminal NH$_3$ concentrations observed after the intraruminal administration of the live brewer’s yeast slurry indicated that the yeast cells were extensively lysed in the rumen. Microscopic examination of whole ruminal fluid at various times after dosing showed numerous cell fragments and the extrusion of cytoplasmic contents from partially intact yeast cells. Because the live brewer’s yeast slurry had been stored for about 30 d at a room temperature, an increase in the NH$_3$ content of the slurry may have contributed to the higher ruminal NH$_3$ concentrations. However, there was no microscopic evidence to indicate that extensive autolysis had occurred in the live brewer’s yeast slurry before administration. The live brewer’s yeast slurry was about 16% viable when administered, and dead cells were probably more predisposed to lysis than live cells. The data indicated that killed brewer’s yeast also underwent lysis in the rumen. However, ruminal NH$_3$ concentrations after dosing with killed brewer’s yeast slurry were less than half the observed NH$_3$ concentrations after dosing with live brewer’s yeast slurry.

The difference in ruminal NH$_3$ concentrations between the live and killed brewer’s yeast slurries could not be explained by the difference in DM percentage of the slurries or by a mass action effect. The killed brewer’s yeast slurry had higher DM concentrations but lower ruminal NH$_3$ concentrations, and there was no proportionality between ruminal NH$_3$ concentrations and the amount of yeast crude protein administered. Although a change in rumen volume could have been responsible, the magnitude of the difference meant that rumen volume must have increased by more than twofold to account for such dilution. This was not likely under the conditions of the study. Because live brewer’s yeast slurry contained considerably more ethanol than killed brewer’s yeast slurry, it is possible that ruminal NH$_3$ production may have been stimulated by the additional ethanol. However, Emery et al. (1959) found no effect of a large daily intraruminal dose of ethanol on ruminal NH$_3$ concentrations. If ethanol had either adversely affected NH$_3$ use by the ruminal bacteria or had induced ruminal stasis, ruminal NH$_3$ concentrations also might have increased.

A plausible explanation for the difference between live and killed brewer’s yeast in ruminal NH$_3$ concentrations is that in the heat processing the cell wall and soluble proteins contained in the yeast cytoplasm were denatured, rendering them more resistant to lysis and microbial degradation. It has been established that heat processing decreases the solubility of protein in feedstuffs and reduces the rate of microbial fermentation in the rumen (Ferguson, 1975). Decreased solubility of yeast protein by heat was not directly examined in this study, but photomicrographs showed that yeast cells were morphologically affected by heat processing. Yeast cells have thick cell walls that are relatively resistant to mechanical disruption (McMurrough and Rose, 1967). However, during autolysis, there are losses in amino acids, glucosamine and other cell wall
components (Charpentier et al., 1986). Boiling brewer's yeast cells will prevent autolysis and will stabilize protein losses (Macrae et al., 1942). If heat processing of yeast cells decreases protein solubility, such effect may be of practical advantage for efficient use of brewer's yeast slurry as a feed by suppressing excessive NH₃ levels in the rumen and allowing more escape of yeast protein to the lower gut. Further study is needed to test this hypothesis.

**Literature Cited**


