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EFFECTS OF AUTOLYZED YEAST (*SACCHAROMYCES CEREVISIAE*) ON FEED
DEGRADABILITY AND DIGESTIBILITY; PERFORMANCE, RUMEN ENVIRONMENT,
AND PHYSIOLOGICAL BIOMARKERS OF HOLSTEIN COWS FED A HIGH STARCH
DIET

BY

SARA ELIZABETH KNOLLINGER

THESIS

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Master's Committee:

Associate Professor Felipe C. Cardoso, Chair
Professor Emeritus Michael R. Murphy
Professor James K. Drackley
Professor Juan J. Lóor

ABSTRACT

High starch diets, because of their increased energy density, are known to enhance animal performance. However, HS diets can have negative impacts on the animal when fed for an extended period, thus reducing animal productivity and performance. The addition of autolyzed yeast (**AY**) has been shown to have beneficial effects while feeding HS diets, resulting in positive effects on animal performance. The objective of the following study were to examine the effects of a commercially-available autolyzed yeast product (Levabon® Rumen E, BIOMIN Holding GmbH, Austria) in HS diets on lactation performance, rumen environment parameters, inflammatory markers, opportunity to improve N utilization, nutrient bioavailability, apparent digestibility, and in situ degradability, gene expression, and the rumen microbiome in lactating Holstein cows. Fifteen multiparous rumen-cannulated Holstein cows were assigned to 1 of 5 treatments in a replicated 5×5 Latin square design balanced to measure carryover effects. Treatments were: low starch diet without AY (**LS0**; control), high starch diet without AY (**HS0**), high starch diet with either 15 g (**HS15**), 30 g (**HS30**), or 45 g (**HS45**) of AY supplementation. Each period (21 d) was divided into an adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). Cows were milked 3 times per d, and samples were collected at each milking on d 15 and 20 of each period. Rumen fluid was sampled on d 15 and 16 starting at time point 0, which was related to feeding at 1400 h. Blood was sampled on d 15, 18, and 21. During the measurement phase, total mixed ration samples were collected on d 18 through 20 andorts on d 18 through 21 and analyzed for dry matter (**DM**), neutral detergent fiber (**NDF**), crude protein, starch, organic matter (**OM**), total N, and undegradable NDF at 120 h (**uNDF 120 h**). Urine samples were collected by manually stimulating urination at 0800, and 2030 h on d 20 of each period. Fecal samples were collected on d 18 through 20, every 8 h for a total of 8 collections per

period. The fecal samples, TMR, and orts were used to calculate total-tract digestibility. In situ degradability determination of a standard alfalfa hay, corn silage (CS), and soybean meal was performed on d 19 through 21 of each period. Rumen papillae and liver biopsies were conducted on d 21 of each period. One single degree-of-freedom contrast LS0 compared to HS0 was used, and linear, quadratic, and cubic treatment effects of HS0, HS15, HS30, and HS45 for statistical inferences. Cows in HS0 had increased dry matter intake (DMI; 24.9 kg/d), milk yield (34.1 kg/d), and energy corrected milk (ECM; 34.4 kg/d) compared to LS0 (19.9 kg/d, 30.9 kg/d, and 31.3 kg/d, respectively). Cows in HS0 had greater protein (1.1 kg/d), casein (0.43 kg/d), and lactose yield (1.6 kg/d) when compared to LS0 (0.94 kg/d, 0.32 kg/d, and 1.4 kg/d, respectively). Cubic treatment effects were present for milk components, AY HS30 tended to increase milk yield, 3.5% fat corrected milk (FCM), lactose yield, and increased ECM, protein and casein percentage, while improving MUN. The cows in LS0 had greater FCM/DMI (1.6), ECM/DMI (1.6), and milk/DMI (1.6) efficiencies when compared to HS0 (1.3, 1.3, and 1.4, respectively). Cows in HS45 had increased DMI (25.6 kg/d) compared to cows in HS0 (24.9 kg/d). Cows in HS15 tended to improve 3.5% FCM/DMI (1.4), and ECM/DMI (1.4) efficiencies than HS0 (1.3, and 1.3, respectively). Cows in HS0 had lower ruminal pH (6.1), nadir pH (5.5), and fecal pH (6.7) when compared to LS0 (6.4, 5.7, and 6.9, respectively). Cows in HS0 had greater propionate and decreased acetate concentrations when compared to LS0, and tended to have a cubic treatment effect for acetate concentration. Cows in HS0 had greater N intake (719.6 vs. 583.7 g/d), milk protein N (166.0 vs. 140.3 g/d), and fecal N (268.4 vs. 219.8 g/d) with a tendency for greater microbial N production when compared to LS0. Supplementing AY decreased fecal N excretion, lowest at HS15. Cows in HS0 had greater nutrient intakes for OM (22.6 vs. 18.6 kg/d), crude protein (4.5 vs. 3.7 kg/d), and starch (6.6 vs. 4.7 kg/d) when compared

to cows in LS0. Cows in LS0 had a tendency for greater digestibility for starch (95.0 vs. 93.7 %) and NDF (52.0 vs. 45.7 %), when compared to HS0, AY increased starch and NDF digestibility. Cows in LS0 had a greater degradable fraction of OM (0.35 vs. 0.28), NDF (0.75 vs. 0.37), and acid detergent fiber (0.66 vs. 0.43) for CS when compared to HS0. For CS NDF k_d and acid detergent fiber degradable fraction tended to increase with AY, while undegradable starch decreased. There were no differences for LS0 compared to HS0 for liver and rumen papillae gene expression. Yeast supplementation increased expression of *NFKB1* and *TLR4* with increased AY dosage (greatest expression at 0.99 and 1.05, respectively). There were no differences for genes associated with acute phase protein or metabolism associated genes in hepatic tissue. Additionally, for rumen papillae there was a quadratic treatment effect for *CD14*, expressed greatest at HS15 (1.03). There was a cubic treatment effect for *TLR2*, expressed greatest at HS15 (1.00). Tight junction gene *CLDN1* tended to linearly increase expression with increased AY dosage. Cows in HS45 (0.96) had the greatest *CLDN1* expression compared other HS treatments. There were no differences for genes associated with metabolism in rumen papillae. High starch diets increased DMI, milk production, milk protein, casein, and lactose yields, lowered production efficiencies, increased VFA propionate concentration, and produced and excreted more N compared to LS0. Supplementing AY increased DMI, rumen pH, nadir pH, improved production efficiencies, milk components, increased VFA propionate concentration with greater AY dosage, reduced fecal N excretion, improved rate of degradation, and starch digestibility for CS, had greater *CD 14*, *TLR2* and *CLDN1* rumen papillae and *NFKB1* and *TLR4* in hepatic tissue gene expression when compared to HS0. In conclusion, adding AY to a HS diet had many benefits when fed to dairy cattle. This could be an important supplement to be considered by nutritionists that wish to formulate HS diets for dairy cows.

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“If you want to be happy, set a goal that commands your thoughts, liberates your energy and inspires your hopes.” - Andrew Carnegie

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
AFRC	Agricultural and Food Research Council
AH	Alfalfa hay
AOAC	Association of Analytical Communities
AST	Aspartate aminotransferase
AUC	Area under the curve
AY	Autolyzed yeast
BCS	Body condition score
BHB	β -hydroxybutyrate
BUN	Blood urea nitrogen
BW	Body weight
Ca	Calcium
CD 14	Cluster of differentiation 14
CHO	Carbohydrate
CLDN1	Claudin- 1
CON	Control
CONT	Contrast
CP	Crude protein
CPK	Creatine phosphokinase
CS	Corn silage
Cu	Copper
D	Digestible fraction
d	Day(s)
dL	Deciliter
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ECM	Energy corrected milk
ED	Effective degradability

EDTA	Ethylenediaminetetraacetate
ELISA	Enzyme-linked immunosorbent assay
FCM	Fat corrected milk
Fe	Iron
g	Gram
<i>g</i>	Gravity
GC	Ground corn
GGT	Gamma-glutamyl transpeptidase
GLDH	Glutamate dehydrogenase
GSH-Px	Glutathione peroxidase
h	Hour(s)
HCL	Hydrochloric acid
HMC	High moisture corn
HRP	Horseradish peroxidase
HS	High starch
HS15	High starch with 15 g of yeast
HS30	High starch with 30 g of yeast
HS45	High starch with 45 g of yeast
K	Potassium
K _d	Fractional digestion rate
K _p	Assumed fractional rate of passage
kg	Kilogram(s)
L	Liter
LBP	Lipopolysaccharide binding protein
log10	logarithm (base 10)
LPS	lipopolysaccharides
LS	Low starch
LSM	Least squares means
ME	Metabolizable energy
MFD	Milk fat depression
Mg	Magnesium

mg	Milligram(s)
mL	Milliliter
mmol	Millimole (mass)
μg	Microgram
Mn	Manganese
Mo	Molybdenum
MUN	Milk urea nitrogen
<i>N</i>	Normal (concentration)
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fiber
NDFD120	Neutral detergent fiber digestibility at 120 h
NEFA	Nonesterified fatty acid(s)
NE _L	Net energy of lactation
NFC	Non-fiber carbohydrates
NFKB1	Nuclear factor kappa B1
NSC	Nonstructural carbohydrates
NRC	National Research Council
OM	Organic matter
P	Phosphorus
PD	Purine derivatives
PUFA	Polyunsaturated fatty acids
S	Sulfur
SS:SA	Sample size to bag surface area
SAA	Serum amyloid A
SARA	Subacute ruminal acidosis
SBM	Soybean meal
SCC	Somatic cell count
SCM	Solid corrected milk
SD	Standard deviation
SEM	Standard error of the mean

SF	Soluble fraction
SOD	Superoxide dismutase
TCA	Tricarboxylic acid
TLR	Toll like receptor
TJ	Tight junctions
TJP1	Tight junction protein 1
TMR	Total mixed ration
TNFA	Tumor necrosis factor A
TRT	Treatment
TVFA	Total volatile fatty acids
U	Unit
uNDF	Undigested neutral detergent fiber
VFA	Volatile fatty acids
YC	Yeast culture
Zn	Zinc

INTRODUCTION

Reduction in milk prices can bring economic pressures on dairy farmers. It is important to increase herd efficiency and improve animal productively. A common practice for lactating cows is to feed high starch (**HS**) diets, to provide adequate energy needed for lactation. Typical HS diets for lactating dairy cows are approximately 25 to 32 % of diet DM (Broderick et al. 2002; Oba and Allen, 2003; Dann et al., 2000). High starch diets have been reported to increase DMI and volatile fatty acid (**VFA**) production, and provide additional energy to the animal for maximal milk production. However, when feeding HS diets to dairy cattle, total mixed rations (**TMR**) can be rapidly fermented. Rapid fermentation in the rumen can lead to lower rumen pH, which can induce subacute ruminal acidosis, also known as SARA. This can create a challenge, wanting to increase animal potential while not compromising the ruminal environment, animal welfare, and reproduction abilities (Zebeli et al., 2007). The rumen environment plays a substantial role in animal efficiency and performance. Feeding HS diets can alter rumen pH, VFA profile, and the rumen ecosystem. Low rumen pH, classified as below 5.6, SARA occurs between a pH of 5.2 and 5.6 for at least 3 h per day (Gozho et al., 2005). Low ruminal pH changes the VFA profile, along with the microbiome of the rumen. Research has reported feeding HS diets increases propionate concentration due to increased energy availability while feeding more forage-based diets are more prevalent in acetate concentration. The rumen is a complex microbial ecosystem, feeding diets high in concentrate predominately decreases the abundance of cellulolytic bacteria such as *Fibrobacter succinogenes* but instead increases the prevalence of *Streptococcus bovis*, common with feeding HS, which can cause a cascade of other changes in the rumen

There is an immense variety of factors in the rumen (pH, microbial population) that can change when feeding HS diets; this can effect animal productivity and efficiency, for example

effecting whole-tract digestion and degradability of feeds. When starch is high and rumen pH is low, it can effect forage digestion (Agle et al., 2010). Studies have reported that diets high in concentrate may decrease the apparent neutral detergent fiber (**NDF**) digestibility (Yang et al., 2001; Beckman and Weiss, 2005; Moorby et al., 2006). Diets high in starch when compared to low starch (**LS**), may have a faster fractional rate of ruminal DM, which would decrease the digestion time (Wiedmeier et al., 1987).

So, why not feed lower starch diets? Although, feeding LS diets can prevent reduction of ruminal pH, feeding lower amounts of starch can negatively affect milk yield and animal performance (Ferraretto et al., 2011). Furthermore, starch concentration in the diet may affect microbial protein production and nitrogen (**N**) utilization in the rumen because energy concentrations and nutrient composition differ (i.e., they vary in nonstructural carbohydrate; **NSC**)

Feed additives are commonly used, in particular yeast to help alleviate the effects from a HS load. The inclusion of autolyzed yeast (**AY**; *Saccharomyces cerevisiae*) in ruminant diets is thought to improve DMI, rumen pH, rumen VFA profile, and nutrient digestibility, which can increase performance and overall animal productivity (Callaway and Martin, 1997; Desnoyers et al., 2009; Dias et al., 2018). However, the concentration of product needed to achieve the most benefit is not well studied, a negative control, such as LS, is often not used, and additionally, oftentimes different feed ingredients are used between diets which confounds the results.

Therefore, the objectives of the following study were to examine the effects of a commercially-available autolyzed yeast product (Levabon® Rumen E, BIOMIN Holding GmbH, Austria) in HS diets on lactation performance, rumen environment parameters, inflammatory markers, opportunity to improve N utilization, nutrient bioavailability, apparent digestibility, and

in situ degradability, gene expression, and the rumen microbiome in lactating Holstein cows. We postulated that the addition of AY supplementation would elicit changes in the rumen that may aid in better rumen conditions thereby, improving lactation performance, efficiency of microbial N use, apparent digestibility, ruminal degradability, and metabolic gene response in rumen papillae and hepatic tissue, thus, having a positive effect on the immune system.

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CHAPTER I

LITERATURE REVIEW

Feeding Starch for Dairy Cows

Starch Metabolism

Currently, there is no dietary requirement for starch listed; however, the NRC (2001) does suggest the maximum feeding concentration rate for non-structural carbohydrates (**NSC**) should be approximately 30 to 40% of the TMR on a DM basis, to reduce the onset of negative effects from SARA (Nocek, 1997). Furthermore, the NSC fraction of corn silage (**CS**), most byproducts, and grains is reported to be mostly starch (Miller and Hoover, 1998). Starch is composed of two major molecules: amylose and amylopectin. Amylose is a linear polymer made up of α -1-4-D-glucose monomers linked together through alpha (1-4) glycosidic bonds. Amylopectin is a branch polymer consisting of linear chains of α -1-4-D-glucose that have an α -1-6 branch point every 20 to 25 glucose residues (Nocek, 1991). Starch digestion in ruminants requires the action of microorganisms in the rumen and several enzymes to convert starch to glucose. Typical HS diets for lactating dairy cows have between 25 to 32% starch on a DM basis (Broderick et al. 2002; Oba and Allen, 2003; Dann el at., 2014). The rate and extent at which ruminal starch is digested may determine how much starch can safely be added to the diet (NRC, 2001). It is estimated, approximately, 90 % of the starch is fermented in the rumen. Most of the starch that escapes ruminal fermentation is digested in the small intestine or fermented in the large intestine (Ørskov, 1986). The rate of starch fermentation can vary greatly depending on the type of grain and its processing technique. However, feeding non-fibrous carbohydrates (**CHO**) do not aid in saliva production or stimulate rumination and may, in fact, impede fiber fermentation and digestibility. Agle et al. (2010) reported lower apparent NDF digestibility in high concentrate (29.6 % starch in DM) diets when compared to low concentrate (21.3 % starch

in DM). The rate of feeding NSC or non-fiber carbohydrates (**NFC**) in replacement of NDF can alter rumen fermentation, total-tract digestion of NDF and milk fat percentages (Sutton and Bines, 1987; Sievert and Shaver, 1993).

Animal Performance

Other carbohydrates, like starch, provide a source of energy to the animal. The VFAs produced by ruminal fermentation, (acetate, propionate, and butyrate) also provide energy to ruminants. The majority of butyrate is converted to ketone bodies. Ketone bodies are an important source of energy for most tissues in the body. Additionally, butyrate stimulates glucose production by increasing gluconeogenesis in the liver (Owen et al., 2002). Acetate and propionate once absorbed through the rumen wall are transported to the liver, where propionate through entering the tricarboxylic acid (**TCA**) cycle is converted to oxaloacetate, the final products being glucose (Owen et al., 2002). Furthermore, acetate from rumen fermentation can be utilized for the synthesis of milk fat and energy purposes. Thus, increased starch increases glucose production, which can improve animal productivity. Another possible source of glucose production in the liver is from lactate by gluconeogenesis. Lactate production occurs when excess starch is present in the diet. Dairy cows fed high starch diets commonly have greater milk yield, milk components, body weight (**BW**), and in some cases DMI (Broderick et al., 2002; Dias et al., 2018a). Previous research has reported a strong positive correlation between milk protein concentration and ruminal propionate yield (Thomas, 1983). However, in addition to having positive benefits on performance, high concentrate diets can have a negative impact on milk fat, which can induce milk fat depression (**MFD**), or low milk fat syndrome (Bauman and Griinari, 2003). Milk fat concentration can change from 0.9 to 2.5 g/kg without changes in milk yield (Shingfield et al., 2010). Milk fat composition can be directly affected by starch concentration,

NDF, and ruminal digestion rates (Harvatine and Bauman, 2011). Feeding HS diets can impede dietary polyunsaturated fatty acids (**PUFA**) transfer to milk, thus increasing pathways to adipose tissue instead (Cabrita et al., 2007). Furthermore, it is important to keep feed ingredients similar between experimental diets to avoid confounding results. Using different feed ingredients can cause discrepancies between the diets, thus potentially influencing the effect of starch concentration on the animal (Dennis et al., 2018). According to Lechartier and Peyraud (2011), feeding HS and LS diets with varying ingredients and different rates of concentrate degradability (slow, moderate, and rapid) had diverse effects on ruminal pH, fermentation, and milk composition. Oba and Allen (2003) fed HS (31% DM) and LS (21% DM) diets using dry ground corn (GC) and high-moisture corn (HMC). When feeding HS and LS with either GC or HMC all diets had different DM proportions and varying ether extract in the HS treatment. The HS diet with either GC or HMC had varying results for VFA production, with no difference in milk characteristics.

Rumen pH, Inflammation, and VFA Profile

Dairy cattle fed HS diets often experience deleterious effects when fed at high concentration for an extended period. These deleterious effects often lead to low ruminal pH, which can cause laminitis, diarrhea, depress DMI, decreased milk production, and alter milk composition, and in extreme cases liver abscess (Duffield et al., 2004; Gozho et al., 2005; Krause and Oetzel, 2006; Plaizier et al., 2008). Cattle consuming highly fermentable CHO can alter rumen function by rapidly increasing acid production. Increased acid production in the rumen decreases ruminal pH and, if low enough, can elicit sub-acute ruminal acidosis or SARA (Penner et al., 2007). Sub-acute ruminal acidosis can be defined as when rumen pH is between 5.2 and 5.6 for at least 3 h/d (Gozho et al., 2005). Low ruminal pH or SARA for an extended period,

several hours per day, can lead to damage of the rumen epithelium (Dias et al., 2018a). The functions of the rumen epithelial tissue include metabolism, nutrient absorption, and pH regulation, in addition to immune and barrier functions. Damage to the rumen epithelium will hinder cow performance, increase inflammation, and adversely affect health issues, for example, the formation of liver abscesses after the release of toxins passing through the rumen wall. These have been identified as free bacterial lipopolysaccharides endotoxin (**LPS**) after translocation from the rumen into the circulation (Plaizier et al., 2008). The release of LPS endotoxins will elicit inflammation and cause changes in blood metabolites and gene expression.

The rumen epithelium functions as a barrier between external and internal environments. It is composed of different multicellular structures and junctional complexes (Graham and Simmons, 2005; Penner et al., 2011; Stumpff et al., 2011). Tight junctions (**TJ**) located in the central layers function by regulating the polarity of epithelial cells, maintaining the permeability of the epithelial barrier, thus inhibiting the translocation of LPS in addition to other toxins (Graham and Simmons, 2005; Penner et al., 2011). Claudin- 1(**CLDN1**) located in the stratum granulosum is one of the primary proteins associated with barrier function in rumen epithelium (Graham and Simmons, 2005). Tight junctions composited of transmembrane proteins (claudins and tight junction protein 1; **TJP1**) seal adjacent epithelial cells and limit the passage of molecules and ions through the space between cells (Liu et al., 2013). Deviations in the protein expression and distribution of the epithelial TJ barrier can lead to impairment (Heiskala et al., 2001; Schulzke and Fromm. 2009; John et al., 2011). This impairment of TJ causes increased epithelial permeability and elicits “leaky” epithelial barrier, causing an inflammation response to LPS and bacteria translocation (Turner, 2009). Liu et al. (2013) reported high grain diets caused ruminal epithelial damage at the cell level, and protein expression and distribution differences.

Rumen generated LPS can bind with LPS-binding protein (**LBP**) and transfer to the cell surface with the support of cluster of differentiation 14 (**CD 14**; Guo et al., 2017). The LPS-LBP-CD 14 complex then activates the TLR4 signaling pathway, thereby increasing the secretion of inflammatory cytokines (i.e., Tumor Necrosis Factor A; **TNFA**). There can elicit an inflammation response (Guo et al., 2017). Lipopolysaccharide toxins produced as a result of SARA can translocate from the rumen, flowing into the bloodstream and triggering inflammation in the liver, and increasing plasma concentrations of acute phase proteins such as serum amyloid A (**SAA**) and haptoglobin (Chang et al., 2015). Inflammation can impair the hepatocytes and, in particular, mRNA expression of genes related to the immune system (Chang et al., 2015). Toll like receptors (**TLR**), such as TLR4, play a key role in the innate immune system and aid in sensing invading pathogens, both intracellular and outside the cell, and produce inflammatory cytokines (i.e.; Nuclear Factor Kappa B1; **NFKB1**). Toll like receptor 4 mRNA and protein has been shown to increased in the liver with SARA and inflammatory stimuli.

Volatile fatty acids are produced from the fermentation of carbohydrates and are absorbed through the wall of the GI tract. These VFA are the primary energy source for ruminants and provide on average 70 % of the total energy required by the animal. Feeding highly fermentable CHO influences the amount and ratio of VFA produced in the rumen (Bergman, 1990). As starch increases, so does VFA production, due to the fact that most starch is fermented quickly. Furthermore, propionate concentration increases when feeding concentrates at the expense of acetate. Thus, as propionate increases, this can increase gluconeogenesis, which may increase milk production.

The amount of CHO in the diet and VFA production can also influence the ecology of the rumen microbiome. Microorganisms in the rumen function best when it is warm, dark, moist,

anaerobic, and filled with substrates (Bergman, 1990). The rumen contains a broad population of anaerobic bacteria, fungi, and protozoa in addition to other species that have a specific niche. Manipulation of the diet influences the available substrates in the rumen. This impacts the metabolic activities of the microorganisms and fermentation end products (Bergman, 1990). Dairy cattle that are fed HS diets are more prone to have bacteria like *Ruminobacter amylophilus* and *Succinomonas amylolytica* in the rumen versus feeding LS diets in which fibrolytic bacteria are more prevalent; for example, *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* (Russell and Rychlik, 2001).

Nitrogen Excretion and Apparent Digestibility

Increased environmental regulations have created new pressures for improving nutrient use by the agricultural industry (Powell et al., 2010). The release of nitrogen (N) in the environment can negatively affect human health through the combination of ammonia and other chemicals in the atmosphere (Powell et al., 2010). Therefore, the excretion of N is becoming more of a concern on commercial dairy farms. Carbohydrate type and the amount fed to dairy cows can have an impact on the quantity and form of N excretion, whether fecal or urinary (Weiss et al., 2009). Previous studies, by Broderick (2003) and Rius et al. (2010) reported that increased consumption of NSC, predominantly corn starch, increased: milk yield, milk protein yield, and N efficiency of lactating cows. Greater starch inclusion in the diet has been reported to increase N intake, milk N, fecal N, and DMI (Moorby et al., 2006). In addition, cows consuming a HS0 diet had greater dietary protein nutrient intakes, which can increase conversion of rumen degradable protein (**RDP**) into microbial protein, thus increasing N utilization efficiency and rumen microbial protein (Sommerfeldt and Lyon, 1988).

Increasing the nutrients and energy digested can improve performance by increasing milk production and profit for the dairy producer. Digestibility by measuring the consumption of feed to what exits the animal's body can help determine nutrient absorption. Digestibility can be measured both directly and indirectly. Direct measurements involves documenting feed intake and fecal excretion of the animal in relation to dietary treatments in a given timeframe (Safwat et al., 2015). A disadvantage of the direct method can be accuracy and contamination of output from the animal. Alternatively, the indirect method requires adding something to the feed, such as metal oxides, or radioactive isotopes that acts as external marker (Nieves et al., 2008; Bovera et al., 2012). The advantage to this method may be increased efficiency, decreasing labor. It is known that as starch concentration increases, replacing forage, the apparent digestibility of fiber (i.e.; NDF and ADF) decreases (Putnam and Loosli, 1959; Tyrrell and Moe, 1972; Valadares et al., 2000). Furthermore, starch digestibility is increased as the NDF to starch ratio increases (Firkins et al., 2001; Beckman and Weiss, 2005). Fecal starch analysis is a technique that can be used to determine apparent starch digestibility (Fredin et al., 2014). Unlike other nutrients (i.e.; protein and fat) that require an internal marker, starch digestibility can be determined by the starch content in feces. The recommended fecal starch percentage should be less than five; however, some suggest less than three (Fredin et al., 2014). If fecal starch is above five percent of fecal DM there is opportunity to improved starch digestibility. Factors that impact starch digestibility are: particle size, corn processing, and fermentation and moisture content. From the cow perspective it is affected by: rumen environment, starch concentration, feeding time, and fractional rate of passage. Techniques to determine total-tract starch digestibility in situ or in vivo, can help in determine the amount of starch the animal utilizes; which is especially important, with increases in cereal grain prices (Firkins et al., 2001). Ruminant starch retention

time can range from 3 to 33 h, based on the type of feedstuff. Goeser (2014) reported in situ starch digestibility at 3, 7, and 16 h for different feedstuffs and determined corn silage starch digestibility averaged 60 to 70% at 3h, 70 to 80% at 7h, and 85 to 95% 16h, with goals of greater than 80, 85, and 95, respectively. The increase in starch digestibility due to increased energy availability can increase animal performance and efficiency (i.e.; milk yield and feed efficiency).

In Situ Degradability

In situ study techniques allow conclusions to be made about ruminal degradation of feedstuffs. However, rumen in situ degradability results can vary depending on a number of factors: bag material, pore size, sample size, sample processing, sample size to bag surface area (**SS:SA**), and animal diet; in addition to overall experimental procedures (Vanzant et al., 1998). A standardized method has been developed to minimize this variation and improve precision within and among laboratories (Vanzant et al., 1998). This method is used to calculate the soluble fractions, degradable fractions, undegradable fractions, effective degradability (**ED**), and fractional rate of degradation (**ka**) of feedstuffs. The recommended incubation times are 0, 2, 4, 8, 16, 24, and 48 and 72 when forages are included (NRC, 2001). Vanzant et al. (1998) recommend the use of 10 mg of DM/cm² for the SS:SA ratio. The particle size of feedstuff in the rumen is also known to influence the rate of passage and rate of digestion (Galyean et al., 1979). Furthermore, the grind size of the feed material can interact with the effect on feed disappearance. It is suggested that a 2-mm screen be used for routine in situ analysis of all feed types (Vanzant et al., 1998). Whereas, the Agricultural and Food Research Council (**AFRC**) recommends a 4 mm grind screen for forages and a 2.5 mm one for concentrates. Additionally, because the type of diet can influence rumen in situ degradation results, in situ incubations

should be conducted in ruminant animals in which the diet consumed is of interest (Vanzant et al., 1998).

Lindberg (1981) and Nocek (1988) reported that diet composition, in particular varying starch content effects on the ruminal microbial population, may affect the extent of degradation, fractional rate of degradability, or both. Cows consuming diets that are high in starch will decrease ruminal pH, causing shifts in the VFA profile and microbiome. These changes can elicit variations in the rumen undegradable and degradable fraction of the diet. Additionally, a more favorable rumen environment (increased pH and saliva production), the forage mat in the rumen, and abrasive action during fermentation may aid in clearing foreign material from pores thus increasing degradation of available substrates (Vanzant et al., 1998). Previous studies have reported in situ forage degradability for animals consuming concentrates decreases compared to animals on a more forage based diet (Lindberg, 1981; Cronje, 1992).

Yeast Cell Wall as a Feed Additive

Yeast Cell Wall vs. Live Yeast

As the agricultural industry advances, livestock producers are seeking alternative to the use of antibiotics and antimicrobials while still promoting animal performance. Live yeast and yeast based products supplemented in the diet of cattle, swine, lambs, and poultry have been reported to have numerous beneficial effects, in particular the *Saccharomyces cerevisiae* strain (Broadway et al., 2015). Around for centuries, *S. cerevisiae* has been used, but not limited to the bread-making and brewing industry (Broadway et al., 2015). Different types of yeast products are defined based on their active ingredients and mode of action. Active live yeast is classified as fermentable, dried, and contain at least 15×10^9 live yeast cells per gram (Alugongo et al., 2017).

Yeast cultures (**YC**) are fermented cereal grains in a certain liquid with bakers yeast, then the whole medium culture is dried. The YC products may also include yeast cell wall (Poppy et al., 2012; Lynch and Martin, 2002). Yeast cell wall products can have antioxidants properties and can be defined as having both probiotic and prebiotic components (Babincová et al., 1999; Kogan et al., 2005; Alugongo et al., 2017). Active live yeast are known to have mainly probiotic effects. Both types of yeast have been reported to have beneficial effects on the immune system, thereby negating the deleterious effects associated with disease and stress (Broadway et al., 2015). Yeast cells intracellular chemical composition include peptides, CHO, salts, amino acids, nucleic acids (**RNA**), enzymes, and cofactors (Maheshwari et al., 2010; Hassan, 2011). Yeast cell wall is derived from one major component, polysaccharides; such as α -D-glucan and β -D-glucan (Kogan and Kocher, 2007). These polysaccharides can interact with immune cells, binding to bacteria and inhibiting the attachment and establishment of pathogens in the gastrointestinal tract (Ruiz-Herrera, 1992). The combination of these properties make them beneficial as a nutritional supplement to livestock, but also as nutraceuticals.

Animal Performance

Providing livestock producers with a “clean label” when feeding natural yeast products that could replace antibiotics and, importantly, increasing performance while decrease morbidity and improving overall profitability (Broadway et al., 2015). Yeast is commonly included in diets for high producing dairy cows because it helps maximize production while migrating the negative effects of a high starch load. The high availability of prebiotic bioactive nutrients in yeast, particularly the yeast cell wall, can modulate the immune response in animals through priming the adaptive and innate immune response, activating immune cells (Li et al., 2005; Volman et al., 2008). A plethora of studies have been conducted studying the effects of yeast

supplementation on various topics involving production and performance of dairy cattle. A YC product meta-analysis of their effects on milk production and other performance measurements in lactating dairy cows concluded that significant improvements in milk production variables such as milk yield, milk fat, and milk protein yield were present (Poppy et al., 2012). Similarly, Wohlt et al. (1998) reported increased DMI and milk yield in early lactating cows. Furthermore, Dias et al. (2018a) found that the addition of yeast increased milk yield, ECM, fat, and milk component yields, as well as ECM/DMI when compared to the control diet. *S. cerevisiae* in ruminant diets improved rumen pH, rumen VFA profile, and nutrient digestibility (Callaway and Martin, 1997; Desnoyers et al., 2009; Dias et al., 2018b).

Rumen pH, Inflammation, and VFA profile

In regard to the rumen environment activity, yeast may elicit a buffering effect by changing the VFA profile in the rumen and increasing ruminal pH. If this is the case, yeast is responsible for reducing ruminal lactate concentration, and promoting lactation performance and animal productivity (Dias et al., 2018a). Supplementation of yeast and increased ruminal pH can increase the fiber-adhering cellulolytic bacteria that can aid in promoting fiber digestion and growth of rumen bacteria (Callaway and Martin, 1997; Neubauer et al., 2018). For dairy and beef cattle supplemented with yeast, a meta-analysis across 110 papers, 157 experiments, and 376 treatments reported that live or dead yeast supplementation increased rumen pH (+ 0.03 on average), rumen VFA concentration (+ 2.17 mM on average), and reduced in rumen lactic acid concentration (-0.9 mM on average), indicating an improvement in rumen fermentation (Desnoyers et al., 2009; Ishaq et al., 2017). The positive benefits of yeast on the immune system may mitigate the inflammation response when cattle are fed high starch diets for an extended period. Dias et al. (2018b) reported that when dairy cows were fed HS diets and supplemented

with yeast plasma haptoglobin concentrations decreased. Haptoglobin plasma alterations suggest that the hepatic tissue was easily changed by proinflammatory mediators induced by diet change. Thus, suggesting supplementing yeast to cows fed HS diets attenuated the inflammatory response. Beta-glucan in yeast cell walls, according to Li et al. (2005), can mitigate the increase of proinflammatory cytokines such as TNFA. It has been reported yeast supplementation may reduce the incidence of liver abscesses from LPS in animals fed HS diets (Garcia Diaz et al., 2018). However, inflammation helps the body overcome adverse challenges and aids in adaption, with the objective of restoring homeostasis (Bradford et al., 2015). For example, not all inflammation is negative. If there are no classic signs of inflammation (redness, pain, and swelling) and no acute phase protein response then this could just indicate an improved immune function (Bradford et al., 2015). Ruminant VFA patterns shift with the addition of yeast supplementation along with changes in rumen pH and the microbiome. The acetate:propionate ratio seems to shift, decreasing acetate and increasing propionate (Harrison et al., 1988).

Nitrogen Excretion and Apparent Digestibility

Rumen stabilization may improve degradability and increase nutrient utilization. In addition, the inclusion of feed additives, such as yeast, has been shown to improve animal productivity through efficiency and digestion. Improved digestion efficiency increases the rate at which gross energy converts to digestible energy. Increasing the nutrients and/or energy digested can aid in improved performance by increasing milk production and profit for the dairy producer. Yeast supplementation properties may interact with the N concentration in the diet (Moloney and Drennan, 1994). According to Julien et al. (2015), yeast supplementation, in particular the *S. cerevisiae* strain, may reduce N excreted in feces and reduce CH₄ mitigation based on microbiome changes in the rumen (Lascano et al., 2012; Dias et al., 2018b; Jin et al., 2017).

Moreover, in vitro research has suggested YC supplementation can increase the utilization of H₂ by acetogens, thus decreasing CH₄ production (Chaucheyras et al., 1995). Yeast supplementation may modulate changes in the rumen, thus increasing fibrolytic bacteria that have a high inclination for ammonia as an N source (Bryant, 1973). If this is the case then increased utilization of ammonia in the rumen for synthesis of microbial protein may be expected, improving overall dietary N efficiency (Hristov et al., 2010). A decrease in fecal N excretion by AY supplementation can have a positive impact on the environment (Powell et al., 2010; Hernandez et al., 2017). Many studies have reported improved OM digestibility when *S. cerevisiae* was supplemented because of increased fiber digestion (Desnoyers et al., 2009; Lascano et al., 2012). Additionally, CP digestibility is increased with YC supplementation (Wohlt et al., 1991; Erasmus et al., 1992; Mwenya et al., 2005; Dias et al., 2018a).

In Situ Degradability

There is little research about how yeast supplementation affects in situ degradability. The changes that can occur in the rumen with yeast supplementation, like improved ruminal pH, microbial ecology, and VFA profile will influence in situ degradability. Yeast supplementation of ruminant diets has been reported to not affect in situ degradation of DM or fiber (Erasmus et al., 1992; Mir and Mir, 1994; Doreau and Jouany, 1998). Gomes et al. (2010) and Meller et al., (2019) reported no effect of yeast supplementation on in situ degradability.

Conclusions and Objectives

In conclusion, it is challenging for a nutritionist to differentiate between the various yeast products and their mode of action in regards to animal performance. It has been widely reported yeast derived products enhance health and immunity during stressful times and negate harmful effects associated with disease and morbidity. However, each commercial yeast may have its

specific recommendations, and there is no standard procedure by the U.S. or global feed industry used. Thus, the objectives of the following study were to examine the effects of a commercially available autolyzed yeast product (Levabon® Rumen E, BIOMIN Holding GmbH, Austria) on:

1. Production parameters and inflammatory metabolites,
2. Improvement of N utilization, nutrient bioavailability,
3. Apparent digestibility and in situ degradability, and
4. Liver and rumen papillae tissue gene expression and the rumen microbiome.

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CHAPTER II

AUTOLYZED YEAST SUPPLEMENTATION REDUCES THE NEGATIVE EFFECTS OF A HIGH STARCH DIET ON RUMEN HEALTH AND PRODUCTIVE VARIABLES OF LACTATING HOLSTEIN COW

ABSTRACT

High starch (**HS**) diets fed for an extended period may reduce rumen pH and shift its volatile fatty acid profile, resulting in potential health problems and reduced performance in dairy cows. The addition of autolyzed yeast (**AY**) has proven to have beneficial effects while feeding HS diets, resulting to positive impacts on production parameters. Fifteen multiparous rumen-cannulated Holstein cows were assigned to 1 of 5 treatments in a replicated 5×5 Latin square design balanced to measure carryover effects. Treatments were: low starch diet without AY (**LS0**; control), HS diet without AY (**HS0**), high starch diet with either 15 g (**HS15**), 30 g (**HS30**), or 45 g (**HS45**) of AY supplementation. Each period (21 d) was divided into an adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). Cows were milked 3 times per d, and samples were collected at each milking on d 15 and 20 of each period. Rumen fluid was sampled over time on d 15 and 16. Blood was sampled on d 15, 18, and 21. One single degree-of-freedom contrast LS0 compared to HS0 was used, and linear, quadratic, and cubic treatment effects of HS0, HS15, HS30, and HS45. Cows in HS0 had increased dry matter intake (DMI; 24.9 kg/d), milk yield (34.1 kg/d), and energy corrected milk (ECM; 34.4 kg/d) compared to LS0 (19.9 kg/d, 30.9 kg/d, and 31.3 kg/d, respectively). Cows in HS0 had greater protein (1.1 kg/d), casein (0.43 kg/d), and lactose yield (1.6 kg/d) when compared to LS0 (0.94 kg/d, 0.32 kg/d, and 1.4 kg/d, respectively). Cubic treatment effects were present for milk components, AY HS30 tended to increase milk yield, 3.5% fat corrected milk (FCM), lactose yield, and increased ECM, protein and casein percentage, while improving MUN. The cows in LS0 had greater

FCM/DMI (1.6), ECM/DMI (1.6), and milk/DMI (1.6) efficiencies when compared to HS0 (1.3, 1.3, and 1.4, respectively). Cows in HS45 had increased DMI (25.6 kg/d) compared to cows in HS0 (24.9 kg/d). Cows in HS15 tended to improve 3.5% FCM/DMI (1.4), and ECM/DMI (1.4) efficiencies than HS0 (1.3, and 1.3, respectively). Cows in HS0 had lower ruminal pH (6.1), nadir pH (5.5), and fecal pH (6.7) when compared to LS0 (6.4, 5.7, and 6.9, respectively). Cows in HS0 had greater propionate and decreased acetate concentrations when compared to LS0, and tended to have a cubic treatment effect for acetate concentration. In conclusion, the addition of AY improved DMI, milk components, production efficiencies, and rumen pH when cows were fed a HS diet.

Keywords: starch, yeast, efficiency, rumen pH

INTRODUCTION

Increasing dietary starch concentration by feeding readily fermentable grains can mitigate negative energy balance postpartum and improve cow performance. Typical high starch (**HS**) diets for lactating dairy cows are approximately between 25 to 32 % of diet DM (Broderick et al. 2002; Oba and Allen, 2003a; Dann et al., 2014). Dairy cattle often experience deleterious effects when fed highly fermentable diets for an extended period, exposing cows to a greater risk of ruminal acidosis (Penner et al., 2007). Diets high in fermentable concentrates can alter rumen function, leading to increased acid production as well as reduced rumination and salivation, and result in decreased rumen pH and potential inflammation of the rumen epithelia (Dias et al., 2018b; Shi et al., 2019). Reduced dietary starch increases ruminal pH and acetate concentration when compared to HS diets (Ferraretto et al., 2011). However, feeding reduced starch diets may result in decreased milk yield.

When examining cow response to the different effects of feeding HS and low starch (**LS**) treatments, dietary composition should remain similar. Using different feed ingredients can cause

discrepancies between the diets, potentially influencing the effect of starch concentration on the animal (Dennis et al., 2018). According to Lechartier and Peyraud (2011), feeding HS and LS diets with varying ingredients and different rates of concentrate degradability (slow, moderate, and rapid) had diverse effects on ruminal pH, fermentation, and milk composition. Oba and Allen (2003a) fed HS (31% of starch DM) and LS (21% of starch DM) diets using dry ground corn (**GC**) and high-moisture corn (**HMC**). When feeding HS and LS with either GC or HMC all diets had different DM proportions and varying ether extract in the HS treatment. The HS diet with either GC or HMC had varying results for VFA production (moles/d), with no difference in milk composition.

A mitigation practice to help offset the negative effects of a HS diet is the supplementation of yeast. Yeast is commonly included in diets for high producing dairy cows because of its positive effects on maximizing production. In addition to the high availability of prebiotic bioactive compounds, including β -glucans and nutrients in autolyzed yeast (**AY**), other components of the yeast cell wall have been found to modulate the immune system in animals by priming the immune response by activation of immune cells (Li et al., 2005; Volman et al., 2008). Poppy et al. (2012) reported that cows early in lactation (<70 DIM) have increased benefits from yeast culture (**YC**) supplementation compared to later lactation cows (≥ 70 DIM). Feeding YC in early lactation can help mitigate health problems by maintaining DMI. It has been well documented that inclusion of AY (*Saccharomyces cerevisiae*) in ruminant diets improves DMI, rumen pH, rumen VFA profile and nutrient digestibility (Callaway and Martin, 1997; Desnoyers et al., 2009; Dias et al., 2018b). Supplementation of AY in highly fermentable diets may reduce ruminal lactate concentration, thus increasing ruminal pH and promoting lactation performance (Dias et al., 2018a). Additionally, AY may increase the fiber-adhering cellulolytic

bacteria that promote fiber digestion and growth of rumen bacteria (Callaway and Martin, 1997; Neubauer et al., 2018). Together, these findings indicate that AY supplementation to dairy cow diets could effectively enhance cellulolytic activity and thereby improve the rumen environment. However, the mode of action, effects from diet composition effect, and recommended concentration are not well understood and can vary among yeast products.

Therefore, the objectives of this study were to determine the effects of supplementation of commercially available AY in high starch diets on lactation performance, rumen environment parameters, and inflammatory markers in lactating, Holstein cows. This study postulated that the addition of an AY supplementation may improve the rumen environment when feeding HS diets, thus aiding in lactation performance.

MATERIALS AND METHODS

Animal Care and Housing

All experimental procedures were approved by the University of Illinois (Urbana-Champaign) Institutional Animal Care and Use Committee (#17172). The experimental period was from January through April 2018. Cows were housed in tie stalls with sand bedding, fed ad libitum, and had free water access. Diets (TMR) were formulated using AMTS.Cattle.Pro version 4.7 (2017, AMTS, LLC, Groton, NY) to meet or exceed recommendations (Table 1) for cows at 70 DIM, 703 kg of BW, producing 41 kg of milk/d with a target of 3.8% milk fat and 3.2% milk protein, and a predicted DMI of 25 kg/d.

Experimental Design

A total of 15 multiparous rumen-cannulated Holstein cows at the start of the experiment [BW (mean $\pm$ SD) = 623 $\pm$ 73 kg; DIM = 77 $\pm$ 26] were assigned to 1 of 5 treatments in a replicated 5 $\times$ 5 Latin square design balanced to measure carryover effects. Therefore, treatments

were arranged so that the carryover effects could be evaluated. Periods (21 d) were divided into an adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). On d 1 to 21 of each period cows received 1 of 5 dietary treatments: LS diet without autolyzed yeast (**LS0**) or HS diet without AY (**HS0**), 15 g of AY (**HS15**), 30 g of AY (**HS30**), or 45 g of AY (**HS45**). The AY (*S. cerevisiae*) product was spray-dried and produced by an internal process technology for standardized autolytic degradation of the yeast cell (Levabon Rumen E, BIOMIN Holding GmbH, Austria). All cows were fed their respective diets once daily at 1400 h throughout the trial. The daily AY allocation was mixed with 300 g of ground corn and top-dressed onto the TMR immediately after feeding. Cows in LS0 and HS0 were given a top dress consisting of 300 g of ground corn only. The total consumption of the top dress was verified daily.

Data Collection and Sampling Procedures

Samples of TMR were obtained weekly and analyzed for DM (AOAC, 1995a) by drying for 24 h in a forced-air oven at 110°C. Diet composition was adjusted weekly for changes in DM content of ingredients. The TMR offered and refused from each cow was recorded to determine intake based on weekly DM analyses. Total mixed ration samples were gathered weekly (3 per period) and stored at –20°C until analyzed. Composite samples for each period (n = 5) were analyzed for contents of DM, CP, ADF, NDF, lignin, NFC, starch, fat, ash, Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, and S using wet chemistry methods (Dairy One, Ithaca, NY). Values for NE_L were provided by the lab and calculated based on NRC (2001). The physical characteristic of the TMR, based on the Penn State Particle Separator (Kononoff et al., 2003), was performed weekly.

Cows were milked 3 times daily at 0400, 1200, and 1930 h. Milk weights were recorded at every milking and samples were obtained at each milking on d 15 and 20 of each period. A preservative (800 Broad Spectrum Microtabs II; D&F Control Systems, Inc., San Ramon, CA)

was added to the samples collected on d 15 and 20 and preserved samples were stored in a refrigerator at 8°C until they were composited in proportion to milk yield and sent to a commercial laboratory (Dairy One, Ithaca, NY) to be analyzed for contents of fat, true protein, MUN, lactose, total solids, casein, and for somatic cell count (SCC) using mid-infrared procedures (AOAC, 1995b).

Rumen fluid was collected via rumen cannula with a syphon on d 15 and 16 after feeding at 1400 h (time point 0). Samples were then extracted at 4, 8, 12, 16, 20, and 24 h after feeding. Rumen samples were collected and composited from 3 sites (ventral sac, cranial sac, and caudo-ventral blind sac). Whole rumen samples were mixed by hand and squeezed through 2 layers of cheesecloth before immediately analyzing rumen fluid for pH (AP115, Fisher Scientific, Pittsburgh, PA). A 20-mL sample of rumen fluid was added to 20 mL of 2N HCL immediately and placed on ice then stored at -20°C until analyzed for VFA concentration. On d 18 and 20 fecal pH was recorded (AP115, Fisher Scientific, Pittsburgh, PA).

Blood was sampled from the coccygeal vein or artery at 0600 h on d 15, 18, and 21 (n = 3) of each period from each cow (BD Vacutainer; BD and Co., Franklin Lakes, NJ). Serum and plasma samples were obtained by centrifugation of the tubes at $2,500 \times g$ for 15 min at 4°C and stored at -80°C for further analysis. Beta-hydroxybutyrate was analyzed from whole blood immediately after sampling using a digital cow-side ketone monitor (Nova Max Plus, Nova Biomedical Corporation, Waltham, MA). Heparinized plasma samples (n = 225) were analyzed for bovine chemistry profiles (creatinine, BUN, total protein, albumin, globulin, albumin:globulin, Ca, P, Na, K, Na:K, Cl⁻, glucose, alkaline phosphate total, aspartate aminotransferase (**AST**), gamma-glutamyl transpeptidase (**GGT**), total bilirubin, creatine phosphokinase (**CPK**), total cholesterol, glutamate dehydrogenase (**GLDH**), HCO₃⁻, Mg,

triglycerides, and anion gap) using the AU680 Beckman Coulter analyzer (<http://vetmed.illinois.edu/vet-resources/veterinary-diagnostic-laboratory/clinical-pathology/>) at the University of Illinois Veterinary Diagnostic Laboratory. Commercially available kits were used to analyze heparinized plasma samples for superoxidase dismutase (**SOD**; intra-assay CV = 6.8%, inter-assay CV = 13.5%), glutathione peroxidase (**GSH-Px**; intra-assay CV = 6.4%, inter-assay CV = 20.0%), and lipopolysaccharide binding protein (**LBP**; intra-assay CV = 5.8%, inter-assay CV = 12.9%). All kits were performed following the manufacturer's instructions.

Superoxidase dismutase activity was identified using Superoxidase Dismutase Assay kit, the dismutation of superoxide radicals generated by xanthine oxidase and hypoxanthine were measured (Cayman Chemical, Ann Arbor, MI; <https://www.caymanchem.com/product/706002>).

Glutathione peroxidase activity was analyzed using Glutathione Assay kit using an indirect approach measuring glutathione reductase for the quantification of glutathione reduction by GSH-Px (Cayman Chemical, Ann Arbor, MI; <https://www.caymanchem.com/product/703102>).

Plasma LBP was measured using the Human Lipopolysaccharide Binding Protein Multispecies Reactive ELISA Kit (Cell Sciences, Newburyport, MA; <https://www.cellsciences.com/human-lbp-elisa-kit-w-precoated-plates>). Commercial kits were used to analyze EDTA plasma samples for haptoglobin, non-esterified fatty acid (**NEFA**; intra-assay CV = 4.5%, inter-assay CV = 11.1%), and serum amyloid A (**SAA**; intra-assay CV = 4.8%, inter-assay CV = 14.9%). Plasma haptoglobin (intra-assay CV = 2.7%, inter-assay CV = 16.9%) was assessed using Cow Haptoglobin ELISA kit in which cow haptoglobin antibodies for solid phase immobilization and horseradish peroxidase conjugated cow haptoglobin antibodies for detection (Life Diagnostics, Inc., West Chester, PA; <https://lifediagnostics.com/shop/elisa-kits/bovine-haptoglobin-elisa/>). Non-esterified fatty acid analysis was performed using a (Wako Diagnostics

U.S.A, Richmond, VA; http://www.wakodiagnostics.com/r_nefa.html) procedure followed by Johnson and Peters (1993). Serum amyloid A was analyzed using the Phase Range Multispecies SAA ELISA kit (Tridelta Development, Ltd., Maynooth, Ireland; <http://www.trideltaltd.com/Serum-Amyloid-A-Assay-Kit.html>). Serum samples were analyzed using the D-lactate Assay kit (Intra-assay CV = 4.5%, inter-assay CV = 15.2%; Cayman Chemical, Ann Arbor, MI; <https://www.caymanchem.com/product/700520>), and Bovine Insulin ELISA kit (intra-assay CV = 4.4%, inter-assay CV = 12.4%; Mercodia AB, Uppsala, Sweden; <https://www.mercodia.se/mercodia-bovine-insulin-elisa>).

Health evaluations and visual assessments were performed for three consecutive days at the start of each period to monitor general appearance and fecal score. Rectal temperature was measured using a GLA M700 Thermometer (GLA Agricultural Electronics, San Luis Obispo, CA). Respiration rate was recorded by visually watching the cow breathe for 15s, and heart rate was measured via palpation of the femoral artery for 15s. General appearance was scored using a similar method to Krause et al. (2009): using a scoring system 1 through 4, 4 = bright and alert; 1 = down cow, without getting up. Fecal scores were allocated on a 1 through 4 scale according to Krause et al. (2009): 1 = runny, liquid consistency; 4 = hard, dry appearance, original form not distorted on impact and settling. Body weight was measured (Ohaus digital scale, model CW-11, Newark, NJ) and BCS was assigned in quarter-unit increments for each cow weekly (Ferguson et al., 1994). Three people independently assigned a BCS score and the average score was used for statistical analysis.

Statistical Analyses

Data collected from d 15 to 21 of each period were analyzed using SAS (v. 9.4, SAS Institute Inc., Cary NC). For production variables and fecal pH the MIXED procedure of SAS was used to model the fixed effects of treatment, square, and period:

$$Y_{ijk} = \mu + T_i + S_j + P_k + \varepsilon_{ijk}$$

where Y_{ijk} = the observations for dependent variables; μ = the overall mean; T_i = the fixed effect of the i^{th} treatment; S_j = effect of the j^{th} square; P_k = effect of the k^{th} period; and ε_{ijk} = the random residual error. For variables measured over time (rumen pH), the MIXED procedure of SAS was used to model the fixed effects of treatment, time, treatment by time interaction, square, and period using the following model:

$$Y_{ijkl} = \mu + T_i + H_j + T_i \times H_j + S_k + P_l + \varepsilon_{ijkl}$$

where Y_{ijkl} = the observations for dependent variables; μ = the overall mean; T_i = the fixed effect of the i^{th} treatment; H_j = the time point effect; $T_i \times H_j$ = the interaction of treatment and time point; S_k = effect of the k^{th} square; P_l = effect of the l^{th} period; and ε_{ijkl} = the random residual error. For both models, cow was the experimental unit and considered as a random effect. The experimental design was balanced to measure carryover effects. Carryover effect was tested and was not present for any variable of interest ($P > 0.29$). One single degree-of-freedom contrast was used LS0 compared with HS0 along with the linear, quadratic, and cubic treatment effects for HS0, HS15, HS30, and HS45. Values reported are least squares means and associated standard errors of the mean. Preliminary measurements, prior to treatment administration, were used as covariates when analyzing the dependent variables DMI, milk yield, BW, and BCS. Area under the curve was calculated based on the incremental area method (Cardoso et al., 2011) with pH = 5.6 as the base line. Degrees of freedom method was Kenward-Rogers (Littell et al., 2002).

Residuals distribution was evaluated for normality and homoscedasticity. A $\log^{10}$ transformation was used for the variables CPK, haptoglobin, SAA, and NEFA. A square root transformation was used for the variable Na in plasma. All transformations were performed for better homogeneity of the distribution of residuals. All means shown for these variables were back-transformed. A multivariable logistic mixed model (FREQ procedure) was used for the dichotomized variables (fecal score, general appearance, and plasma haptoglobin). The chi-square was computed and is presented. General appearance score ≥ 3 were categorized as healthy and ≤ 2 were categorized as abnormal. Fecal score > 2 were categorized as healthy and ≤ 2 were categorized as abnormal (Ireland-Perry and Stallings, 1993; Krause and Øetzel, 2005). Haptoglobin samples were categorized as high ($\geq 31.36 \mu\text{g/mL}$) or low ($< 31.36 \mu\text{g/mL}$) in reference to the median, $31.36 \mu\text{g/mL}$. Statistical significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Diet Composition

The ingredient composition of the diets are in Table 1. Analyzed nutrients from the experimental diets are in Table 2. The physical characteristics of the LS TMR, based on the Penn State Particle Separator (Kononoff et al., 2003) was (mean $\pm$ SD): 3.6 ± 1.4 % on upper (19-mm pore size), 43.2 ± 3.9 % on middle (8-mm pore size), 15.3 ± 1.2 % on lower (4-mm pore size) sieves, and 37.8 ± 4.7 % in the pan. The physical characteristics of the HS TMR, based on the Penn State Particle Separator (Kononoff et al., 2003) was (mean $\pm$ SD) 2.2 ± 0.9 % on upper (19-mm pore size), 32.4 ± 2.6 % on middle (8-mm pore size), 14.3 ± 1.1 % on lower (4-mm pore size) sieves, and 50.6 ± 3.4 % in the pan.

DMI, BW, BCS, and Lactation Performance

Performance data for the measurement phase are in Table 3. We observed no treatment linear effect differences ($P > 0.10$) for any performance variables. Cows in LS0 had lower DMI ($P < 0.001$), BW ($P = 0.003$), and DMI as a percentage of BW ($P < 0.001$) than cows in HS0. We observed a quadratic treatment effect for BW ($P = 0.05$), and a tendency for a cubic treatment effect for DMI ($P = 0.10$). Cows in HS0 had greater milk yield ($P = 0.02$) and ECM yield ($P = 0.03$); and tended to have higher FCM (3.5%) yield ($P = 0.08$) when compared to cows in LS0. We observed a cubic treatment effect for ECM yield ($P = 0.03$), protein yield ($P = 0.01$) and protein percentage ($P = 0.03$), casein percentage ($P = 0.03$), and MUN ($P = 0.03$). We observed cubic treatment tendencies for milk yield ($P = 0.09$), FCM (3.5%) yield ($P = 0.06$), fat percentage ($P = 0.08$), and lactose yield ($P = 0.06$). Cows in the HS0 treatment had greater protein ($P < 0.001$) and casein percentage ($P = 0.008$); along with protein ($P < 0.001$), casein ($P = 0.002$), and lactose yield ($P = 0.004$) compared to LS0. We observed no differences for casein as a percentage of protein, fat yield, or lactose percentage ($P > 0.10$). Cows in HS0 had lower milk fat percentage ($P = 0.007$) when compared to LS0. Cows in the LS0 treatment tended to have greater MUN ($P = 0.09$) compared to HS0. Feed conversion efficiency decreased for cows on treatment HS0 (FCM/DMI, ECM/DMI, and milk yield/DMI; $P < 0.001$, $P < 0.001$, and $P = 0.008$; respectively) compared to LS0. We observed tendencies for quadratic treatment effects with the addition of AY (FCM/DMI, ECM/DMI; $P = 0.09$ and $P = 0.07$, respectively). We observed no difference among treatments for fecal score ($P = 0.4485$), and general appearance health parameters. From 75 fecal score observations, 71 were considered healthy. However, of the 4 cows in the abnormal category, 2 cows were in HS0, 1 in LS0, and 1 in HS30. General appearance of cows observed was considered healthy throughout the experiment. Of the 225

observations for plasma haptoglobin ($P = 0.13$), 206 were classified in the low category, and 19 observations in the high category. Of the 19 cows in the high category, 5 cows were in LS0, 1 in HS0, 4 in HS15, 2 in HS30, and 7 in HS45.

Rumen pH, Fecal pH and VFA

Rumen pH, fecal pH, and VFA response data are in Table 4. Cows in LS0 had greater rumen pH ($P < 0.001$) than cows in HS0. A quadratic effect of treatment was present for rumen pH ($P = 0.04$). Time points differed ($P < 0.0001$) for rumen pH, but we observed no treatment by time point difference ($P = 0.64$; Figure 1). Cows in HS0 had a nadir rumen pH ($P < 0.001$) more acidic than cows in LS0. A positive linear treatment effect was observed for nadir pH ($P = 0.009$). We observed no differences among treatments for cows that spent more time with a ruminal pH below 5.6 ($P > 0.10$). Fecal pH was different between treatments LS0 and HS0, cows in the LS0 treatment had greater fecal pH ($P = 0.042$) when compared to HS0. Cows in HS0 had greater total VFA (**TVFA**; $P < 0.001$) when compared to LS0. We observed a quadratic ($P = 0.06$) and cubic ($P = 0.09$) treatment effects for TVFA. Acetate tended to have a cubic treatment effect present ($P = 0.09$), in addition to a quadratic ($P = 0.02$) treatment effect, as a proportion of TVFA. Cows in LS0 had greater acetate ($P < 0.001$), isobutyrate ($P < 0.001$) and isovalerate ($P = 0.02$) as a proportion of TVFA concentration when compared to HS0. A negative linear treatment effect was present for VFA, as a proportion acetate ($P < 0.001$), isobutyrate ($P < 0.001$) and isovalerate ($P = 0.01$). Cows in HS0 had greater propionate ($P < 0.001$), and valerate ($P < 0.001$) as a proportion of TVFA concentration when compared to LS0. A positive linear treatment effect was present for propionate ($P = 0.002$) as a proportion of TVFA concentration. A quadratic treatment effect was present for butyrate ($P = 0.001$), and a quadratic tendency for isobutyrate ($P = 0.06$). We observed a time ($P < 0.0001$); and treatment $\times$ time ($P < 0.01$)

interaction for acetate as a proportion of TVFA (Figure 2). Propionate as a proportion of TVFA had a time ($P < 0.0001$); and treatment $\times$ time ($P < 0.0001$) interaction (Figure 3). Treatment by day interactions were not present in other variables ($P > 0.33$).

Serum and Plasma Chemistry Profile

Blood plasma and serum chemistry profile are in Table 5. A quadratic treatment effect was present for glucose concentration ($P = 0.03$). A tendency for a negative linear effect was present for BHB ($P = 0.08$), and tendency for a quadratic treatment effect for triglycerides ($P = 0.09$). Cows in LS0 has greater NEFA concentration ($P = 0.04$) in comparison to HS0. Cows in HS0 experienced greater insulin serum concentrations ($P = 0.03$) compared to LS0. Both a linear ($P = 0.03$) and quadratic ($P = 0.02$) treatment effect was present for anion gap. A tendency for a cubic treatment effect was present for D-lactate ($P = 0.07$). Cows in LS0 had greater plasma total protein concentration ($P = 0.03$) when compared to HS0. We observed a quadratic treatment effect for plasma total protein ($P = 0.05$). A tendency for a negative linear treatment effect was present for albumin ($P = 0.08$). A quadratic treatment effect was present for globulin ($P = 0.03$). A tendency for both linear ($P = 0.07$) and quadratic ($P = 0.06$) treatment effects were present for Albumin:globulin ratio. We observed no differences for GLDH, cholesterol, creatinine, CPK, or bicarbonate between treatments ($P > 0.10$). We observed no differences for K, Na: K ratio, or Mg in the blood ($P > 0.10$). Cows in LS0 tended to have greater plasma Ca concentration when compared to HS0 ($P = 0.07$). A quadratic treatment effect was present for Ca ($P = 0.03$). Cows in LS0 had lower P ($P = 0.005$) when compared to HS0. We observed a linear treatment effect ($P = 0.03$) along with a quadratic tendency for Na ($P = 0.07$). A tendency for a quadratic treatment effect was present for Cl ($P = 0.09$). Liver function metabolites AST was not affected by treatment ($P > 0.10$). We observed no liver function plasma biomarkers differences for linear, or

quadratic effects ($P > 0.10$). Cows in HS0 had greater GGT ($P = 0.02$) when compared to LS0. Cows in LS0 had greater total bilirubin ($P = 0.05$) compared to HS0, and a tendency for a cubic treatment effect ($P = 0.08$). A cubic treatment effect was present for total alkaline phosphate ($P = 0.004$). We observed no treatment differences for inflammation biomarkers LBP, or GSH-Px ($P > 0.10$). A tendency for a negative linear effect was present for haptoglobin ($P = 0.06$). We observed a tendency for a linear treatment effect ($P = 0.06$) and a quadratic treatment effect ($P = 0.05$) for SAA. A negative linear treatment effect was present for SOD ($P = 0.05$).

DISCUSSION

This study postulated that the addition of an AY supplementation may improve the rumen environment when feeding HS diets, thus aiding in lactation performance.

This experiment was able to successfully achieve a high ($31.2 \pm 4.2\%$ starch in DM) and a low starch ($22.8 \pm 0.70\%$ starch in DM) diets (Broderick et al. 2002; Oba and Allen, 2003a; Dann et al., 2014). In the current study, corn grain was the main starch source used to achieve a HS diet. When formulating the LS diet we decreased corn grain and increased the corn silage, thus reducing the starch content and increasing NDF. To quantify our diets we considered starch values in the low twenties as LS (< 24) and in the high twenties HS (> 25), on a DM basis for this experiment.

In previous studies, no differences were reported for DMI for cows fed HS and LS concentrations (Ferraretto et al., 2012; Dias et al., 2018b). Broderick et al. (2002) fed mid lactation cows diets containing 31% starch (HS) or 20% starch (LS; TMR DM basis) and the LS diet had reduced DMI. Similar results were observed in the current study. Yeast supplementation at the greater yeast concentrations (HS30, and HS45), tended to improve DMI. Cows in HS45 had the greatest DMI which may explain why cows in the HS45 had the greater BW with AY.

Similarly, Dann et al. (2000) reported an increase in DMI when feeding yeast culture during the transition period. In the current study, cows in the HS0 treatment had greater BW when compared to LS0, and this difference may be attributable to the increase in DMI. Previous research reported that milk yield increases when feeding a HS diet in comparison to LS (Broderick et al., 2002; Dias et al., 2018a). Observed increases in milk yield when feeding HS diets suggest that greater energy concentrations in the diet increase ruminal propionate, providing increased glucose for better utilization of milk yield when compared to LS diets. Additionally, cows in HS30 tended to increase milk yield with AY supplementation. This could be attributable to a combination of factors such as improved ruminal propionate, and pH. A more in depth observation of rumen characteristics will help determine the role of bacteria in the rumen on lactation performance. Desnoyers et al. (2009) conducted a meta-analysis concluding increased milk yield with increased yeast dosage, dissimilar to the current study, at the HS45 dosage milk yield decreased when compared to HS30.

An increase in milk yield for cows in HS0 may be responsible for the observed increase in 3.5 % FCM. Likewise, ECM is elevated in the HS0 diet, and this could be initiated by the greater protein yield. Similarity, this could also explain the increase 3.5% FCM and ECM observed for the HS30 AY supplemented treatment. When comparing milk composition, Dias et al. (2018a) reported no differences between HS and LS diets for yields of 3.5% FCM or ECM, nor with 3.5% FCM/DMI or ECM/DMI feed conversion efficiencies. However, with the addition of yeast Dias et al. (2018a) reported improvements in milk yield, ECM, milk fat and protein yield when compared to the control diet. The present study, reported improvements in milk components (3.5% FCM, ECM, protein and casein percentage, and lactose yield) at the HS30 dosage, but components declined as AY dosage increased. Furthermore, cows in the HS15

treatment improved efficiencies (FCM/DMI, ECM/DMI, and milk yield/DMI) in comparison to the other HS treatments. Feed efficiencies were greater in the LS0 diet compared to HS0. Ferraretto et al. (2011) observed reduced FCM/DMI, ECM/DMI, SCM/DMI, and milk yield/DMI efficiency with reduced dietary starch (30.4 vs. 36.3% starch in DM). Whereas, Gencoglu et al. (2010) found no effect of starch (33.3 vs. 20.9% starch in DM) for the same efficiencies, suggesting that animal responses may vary with dietary ingredients and dietary starch content.

Highly fermentable diets can negatively affect milk composition, particularly milk fat content. In the current study, an increase in milk fat proportion is observed for cows in LS0. Milk fat concentration can be directly reliant on starch concentration, NDF, and ruminal digestion rates (Harvatine and Bauman, 2011). Additionally, increasing starch concentration can decrease the dietary PUFA transfer to milk, thus increasing pathways to adipose tissue instead (Cabrita et al., 2007). However, we observed a tendency for increased fat concentration with AY supplementation for the HS15 treatment. Furthermore, the HS15 diet had the greatest VFA acetate concentration when compared to the other HS diets, contributing to milk fat synthesis. Cows in the LS0 treatment had increased forage NDF in the diet, which allowed for elevated ruminal pH compared to HS0. Furthermore, because cows in HS0 had greater rumen propionate and lower acetate concentration in the rumen this may be associated with suppressed milk fat concentration. A similar mechanism could have occurred for cows in HS0 related to low ruminal pH, as it favors increased propionate, lactic acid, and glucose production, which elevates insulin, thus reducing free fatty acids released from adipose tissue, impacting milk fat (Oba and Allen, 2003b; Ferraretto et al., 2012). Likewise, feeding HS (30% of starch DM) tended to decrease milk fat proportion compared to LS (20 % of starch DM; Ferraretto et al., 2012). Comparable

results were seen in the current study, as cows receiving the HS0 diet had lower concentrations of milk fat.

Another explanation for the decreased fat content for cows in the HS0 diet could be an increased production of trans-fatty acids in the rumen (Jurjanz et al., 1998). Gaynor et al. (1995) reported an increased content of trans-C18:1 in the rumen and in milk fat of diets rich in grain concentrate, which could decrease milk fat content. Both the current study and Dias et al. (2018a) reported greater milk protein proportion and yield for HS diets compared to LS. Increased milk yields and milk protein concentration for cows in HS0 may be attributed to greater energy density of the diet. Previous research has reported a positive correlation between milk protein concentration and ruminal propionate yield (Thomas, 1983). Propionate production generates glucose through gluconeogenesis, once taken up by the mammary gland glucose supports lactose synthesis (Zhao et al., 2014). As a result, we observed greater milk and lactose yield in the HS0 diet. The addition of AY improved protein and lactose yield, and protein and casein percentage for the HS30 treatment, explained by the increased milk yield and ruminal propionate when compared to HS0. Cows in LS0 tended to have increased MUN compared to cows in HS0. Gencoglu et al. (2010) and Ferraretto et al. (2011) reported similar results for MUN concentration. Increasing dietary NDF concentration has been reported to increase ruminal ammonia more so than increasing starch concentration (Ferraretto et al., 2011). This may explain the greater MUN response while feeding the LS0 treatment in the current study. The addition of rapid starch fermentation provided in HS0 may have allowed for increased nitrogen utilization by rumen bacteria, leading to an increase in microbial protein production. Additionally, with AY the HS30 treatment has improved MUN, which may suggest better N utilization. Slightly greater MUN values for the LS0 diet are consistent with reduced milk yield and true protein.

Increasing dietary starch concentration is known to decrease ruminal pH, as was observed in the current study for cows in HS0 compared to LS0 (Radostits et al., 1994). The addition of yeast attenuated shifts in the rumen environment increasing rumen pH, comparable to AlZahal et al. (2014). A similar study conducted by Neubauer et al. (2018) may explain a potential mechanism for the pH stabilization observed when feeding AY. Neubauer et al. (2018) used the same AY product and reported an increase in cellulolytic bacteria, along with the reduction of starch fermenting gram-positive bacteria by yeast when feeding HS concentrates. Nadir pH demonstrated a similar pattern, with HS0 having the lowest pH, and pH increasing as concentration of AY in the diet increased. There are fewer reports in the literature regarding fecal pH. According to Beasom et al. (1982), fecal pH is a function of diet. However, rumen and fecal pH are usually not related unless starch bypasses the rumen and results in hindgut fermentation (Enemark, 2008). According to Gressley et al. (2011), observed variation in fecal consistency, like diarrhea, frothy feces, and mucin casts would occur because of hindgut fermentation. However, in the present study we observed no differences detected for fecal consistency per fecal score evaluation. Previous research has reported that starch fermentation increased propionate concentration while decreasing acetate concentration in the rumen (Rémond et al., 1995; Gao and Oba, 2016). High forage diets stimulate bicarbonate from saliva in the rumen and increase acetate production. This may explain why the LS0 treatment had increased total acetate concentration in the rumen when compared to all other treatments. Reducing physically effective fiber (**peNDF**) in the diet has been reported to increase total rumen VFA concentration (Beauchemin and Yang, 2005). This was observed in the current study, as cows in HS0 had increased ruminal VFA concentration compared to cows in LS0. Propionate concentration may have an effect on DMI, as it can be oxidized in the liver (Oba and Allen, 2003b), decreasing

intakes. If oxidation of propionate in the liver occurred it would be a short term effect and is not likely for the current study as increased ruminal propionate improved DMI. Propionate and butyrate concentrations are most highly correlated to milk yield, which is positively correlated to DMI (Seymour et al., 2005). Cows in HS0 had greater propionate concentrations with greater milk yield and DMI. Gluconeogenic substrates, propionate and valerate both can help synthesize lactose, the main contributor of milk yield (Aschenbach et al., 2010). These data may be supported in the current study, as an increase in milk yield was observed for cows in HS0 compared to LS0.

Supplementation of AY in HS diets increased blood glucose concentration, similar to Lascano et al. (2012). Blood glucose can be an insensitive parameter of energy status because of its homeostatic regulation (Herdt, 2000). Glucose in blood plasma is not used by the liver thus, not a source of energy for ruminant animals (Allen et al., 2009). Similar to Oba and Allen (2003a), cows in HS0 had greater insulin concentrations, which is likely reflective of the increased energy density of the diet compared to LS0. A plasma marker more related to energy status and fat mobilization is NEFA because this metabolite responds more rapidly to changes in energy metabolism (Russel and Wright, 1983). Dias et al. (2018b) reported no treatment effect for HS versus LS for plasma glucose or NEFA. While it is believed that cows in LS0 may be utilizing more fat deposits releasing more free fatty acids, increasing NEFA concentration when compared to the HS0 diet. However, even though cows in HS0 increased BW compared to LS0, the biological meaning of the change is questionable. The increased free fatty acids may be responsible for greater milk fat concentration in cows in LS0. However, the present study did not analyze fatty acids in milk, but further research could be conducted to validate this theory.

Nevertheless, all NEFA concentrations were within the normal range (Cozzi et al., 2011; Piccioli-Cappelli et al., 2014).

Cows in HS0 had lower ruminal pH compared to the addition of AY, which could potentially cause inflammation in the laminae of the hoof leading to laminitis, thus increasing blood CPK (Belge et al., 2004). However, these values are still within the normal range (50 to 320 U/L) and no sign of diseased, or inflamed muscle cells were apparent (Cozzi et al., 2011). Cows in the HS0 treatment experienced greater plasma P concentrations compared to LS0. Feeding HS diets for an extended period of time can initiate rumen acidosis, thus facilitating bone demineralization and causing greater concentrations of plasma P for cows in HS0. However, it is important to report that plasma P concentrations for cows in both LS0 and HS0 treatments were within the normal biological range (3.6-6.9 mg/dL; Cozzi et al., 2011).

Plasma metabolites measured for liver functionality (AST, GGT, total bilirubin and alkaline phosphate) were all within normal ranges (17 to 137 U/L, 4 to 28 U/L, 0.1 to 0.4 mg/dL, 8 to 179 U/L, respectively; Cozzi et al., 2011). Gamma-glutamyl transferase is a membrane bound enzyme located in many organs, such as the liver and can increase during sign of disease that can lead to liver damage (Merck, 2020). Cows in HS0 had greater plasma GGT concentration compared to LS0, which may suggest more GGT passing through the liver when compared to LS0.

A consequence to feeding HS diets is a decrease in rumen pH and the production of bacterial by-products that can prompt inflammation (Dias et al., 2018b). Measuring acute phase protein concentration in the blood, such as albumin, haptoglobin, and SAA, can aid in detecting signs of inflammation. Acute phase proteins are known as proteins that response to inflammation by changing blood concentration by > 25% (ECLINPATH, 2013). Bossaert et al. (2012)

reported that cows with increased inflammation post-calving had decreased albumin concentrations. Similarly Burke et al. (2010) diagnosed cows with endometritis as having decreased plasma albumin. In the current study, the addition of AY in HS diets resulted in decreased plasma albumin concentration with increased AY, having the greatest concentration at HS15, suggesting an innate immune response is occurring with AY. Serum amyloid A concentration was greater for cows in the LS0 treatment compared to cows in HS0, this is surprising given the lower ruminal pH for the HS0 treatment which could potentially lead to inflammation. Thus, the increased SAA for LS0 may imply that some inflammatory event (increase in IL-6 or TNF), might have triggered the SAA response. Additionally, a positive quadratic trend occurred as concentration AY in the HS diet increased, with the greatest SAA concentration occurring for HS15. Elevation of SAA can occur under conditions unrelated to inflammation, such as physical stress (Baghshani et al., 2010). During this study there was no reason to believe cows in any one treatment experienced any additional stress compared to another treatment for the SAA concentrations to increase, as we observed were no differences in fecal scores or general appearance. Cannizzo et al. (2012) reported that medium risk cows for acidosis ($\text{pH } 5.77 \pm 0.35$), had the greatest SAA concentration, while high risk cows with a lower ruminal pH ($\text{pH } 5.57 \pm 0.31$) had a lower SAA concentration. Taken together, these data indicate that increased SAA concentration does not correspond to lower rumen pH values. Likewise, cows at low risk for acidosis ($\text{pH } 6.0 \pm 0.39$) were reported to have the greatest haptoglobin concentration when compared to medium and high risk cows. (Cannizzo et al., 2012). Turlomoussis et al. (2004) determined that cows without pathological conditions had a mean plasma haptoglobin concentration of $20 \pm 0.03 \mu\text{g/mL}$ while the mean haptoglobin concentration in cows with pathological conditions was $27 \pm 0.40 \mu\text{g/mL}$. All cows in this study were without

pathological conditions, as indicated by the mean serum haptoglobin concentration, which was 10.63 µg/mL for cows supplemented with AY.

Oxidative stress marker SOD concentration decreased with increased AY supplementation.

Superoxide dismutase aids in defense against reactive oxygen species, and antioxidant status.

Elevated plasma SOD concentration for cows may indicate physiological upgrading of the enzyme to help stabilize/negate radical challenges, while accumulating animals to oxidative stress in aid of improving antioxidant status (Moolchandani and Sareen, 2018). Increased SOD when feeding HS may be a result of decreased ruminal pH or increased metabolic drive for milk production. High yielding dairy cows with increased milk drive have been reported to have increased production of free radicals and reactive oxygen species (Castillo et al., 2005). Similar to the current study, Abaker et al. (2017) reported that cows fed high grain diets had increased SOD blood concentration than cows fed low grain diets. However, in the current study, since SOD concentration was within normal range, it is likely that minimal oxidative stress occurred.

CONCLUSION

Results from the present study indicated that HS diets increase DMI, and milk yield, while having an impact on milk composition and lowering production efficiencies.

Supplementation of AY improved the rumen environment when feeding HS diets. Additionally, supplementing dairy cow diets with AY increased DMI, milk components, and improved production efficiency. Overall rumen pH and nadir pH were positively affected when adding AY. Propionate concentration in the rumen increased as AY dosage increased. Blood metabolites (NEFA, insulin) indicated that energy metabolism improved for cows in HS0 compared to LS0, with no major implications on inflammation among diets. In the current study, we observed the best AY dosage for improvements on lactation performance and rumen health is at the lower AY

concentrations (HS15, and HS30), and decreased benefits were observed with HS45. Further research, investigating fatty acids in milk, may explain the greater milk fat concentration for cows in the LS0 treatment.

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TABLES AND FIGURES

Table 2.1 Ingredient composition of the diets fed to lactating cows on a low starch (LS) and high starch (HS) diet during the experimental period

Ingredient, % DM	Diets	
	LS	HS
Corn silage ¹	49.77	30.26
Alfalfa hay	16.02	17.28
Soybean meal	13.14	13.38
Dry ground corn grain	6.41	23.23
Canola meal	4.65	5.01
Corn gluten feed	2.69	2.91
Soy hulls	1.87	2.02
Dried molasses	1.38	1.49
Bypass fat ²	1.03	1.11
Dicalcium phosphate	0.40	0.44
Trace mineral ³	0.06	0.07
Rumen protected lysine ⁴	0.06	0.07
Rumen protected methionine ⁵	0.04	0.04
Potassium carbonate	0.13	0.13
Sodium bicarbonate	0.66	0.66
Calcium carbonate	0.65	0.65
Potassium chloride	0.17	0.17
Urea 46%	0.15	0.15
Salt, white	0.07	0.07
Magnesium oxide 54%	0.07	0.07
Vitamin and mineral mix ⁶	0.58	0.78

¹ Corn silage was 29.3 % DM for all treatments.

²Energy booster 100 (Milk Specialties Co., Eden Prairie, MN).

³Availa Dairy (6.67 % Zn, 3.34 % Mn, 0.585 % Cu, 0.167 % Co; Zinpro Corp., Eden Prairie, MN)

⁴Ajipro-L Generation 3 (Ajinomoto Heartland, Inc., Chicago, IL).

⁵Smartamine M (Adisseo, Alpharetta, GA).

⁶Vitamin and mineral mix was formulated to contain 13.50 % Ca, 0.001 % P, 3.92 % salt, 10.90% Na, 6.68 % Cl, 2.33 % Mg, 8.27 % K, 0.14 % S, 1.77 mg/kg Co, 126.98 mg/kg Cu, 32.86 mg/kg I, 602.01 mg/kg Fe, 980.85 mg/kg Mn, 7.47 mg/kg Se, 3.15 mg/kg organic Se, 888.79 mg/kg Zn, 108.86 kIU/kg Vitamin A, 21.77 kIU/kg vitamin D₃, 410.51 IU/kg vitamin E, 2.48 mg/kg choline, 18.21 mg/kg biotin, 0.16 mg/kg Niacin, 0.004 mg/kg thiamine.

Table 2.2 Mean chemical composition and associated standard deviations for low starch (LS) and high starch (HS) diets fed to all cows throughout the experimental period

Item	LS		HS	
	Mean ¹	SD	Mean ¹	SD
DM, %	43.1	2.36	51.9	3.22
CP, % of DM	17.8	0.63	17.2	0.49
ADF, % of DM	21.4	1.37	18.6	1.06
NDF, % of DM	31.8	0.94	28.7	1.24
Lignin, % of DM	3.6	0.41	3.2	0.56
NFC, % of DM	37.5	1.89	42.5	1.52
Starch, % of DM	22.8	0.70	31.2	4.22
Crude fat, % of DM	3.9	0.29	3.8	0.26
Ash, % of DM	8.96	1.73	7.81	1.02
NE _L , Mcal/kg of DM ²	0.74	0.02	0.77	0.02
Ca, % of DM	1.41	0.76	1.07	0.37
P, % of DM	0.44	0.01	0.45	0.01
Mg, % of DM	0.28	0.02	0.27	0.01
K, % of DM	1.57	0.09	1.47	0.04
Na, % of DM	0.33	0.05	0.31	0.01
S, % of DM	0.24	0.01	0.23	0.01
Fe, ppm	402	157.98	354	110.30
Zn, ppm	107	14.41	101	2.47
Cu, ppm	16	1.85	14	0.33
Mn, ppm	101	23.16	88	13.75
Mo, ppm	1.1	0.25	1.1	0.01

¹ Mean diet composition of periods 1, 2, 3, 4 and 5 (n = 5).

² NRC (2001).

Table 2.3 Least squares means and associated SEM for BW, BCS, and production parameters of Holstein cows in low starch diet without autolyzed yeast product (LS0), high starch diet without autolyzed yeast product (HS0), HS with 15 g (HS15), 30 g (HS30), and 45 g (HS45) of autolyzed yeast during the experimental period

Variable	Treatment ¹					SEM ³	P-value Contrasts ²			
	LS0	HS0	HS15	HS30	HS45		LS0 vs. HS0	Linear TRT	Quad TRT	Cubic TRT
DMI, kg/d	19.90	24.88	22.72	24.95	25.56	1.08	<0.001	0.25	0.09	0.10
BW, kg	665	689	671	681	685	8.2	0.003	0.94	0.05	0.14
DMI, % of BW	2.91	3.51	3.46	3.53	3.71	0.25	<0.001	0.20	0.31	1.00
BCS	3.41	3.48	3.47	3.41	3.44	0.06	0.29	0.39	0.68	0.49
Milk yield										
Milk yield, kg/d	30.85	34.08	31.90	34.28	34.10	1.47	0.02	0.55	0.28	0.09
3.5 % FCM kg/d	31.85	34.36	32.30	34.74	33.12	3.13	0.08	0.78	0.82	0.06
ECM, kg/d	31.27	34.39	32.17	34.92	33.20	3.14	0.03	0.85	0.80	0.03
Milk composition										
Fat, %	3.89	3.56	3.78	3.56	3.60	0.17	0.007	0.85	0.30	0.08
Fat, kg/d	1.16	1.18	1.15	1.22	1.16	0.11	0.78	0.96	0.65	0.21
Protein, %	3.13	3.23	3.22	3.29	3.24	0.05	<0.001	0.14	0.23	0.03
Protein, kg/d	0.94	1.10	1.02	1.13	1.07	0.10	<0.001	0.88	0.62	0.01
Casein, %	2.59	2.66	2.65	2.72	2.68	0.04	0.008	0.14	0.51	0.03
Casein, kg/d	0.32	0.43	0.37	0.41	0.41	0.06	0.002	0.81	0.31	0.20
Casein, % of protein	82.06	82.42	82.11	82.59	82.60	0.43	0.29	0.33	0.50	0.23
Lactose, %	4.67	4.73	4.73	4.69	4.72	0.05	0.11	0.56	0.44	0.45
Lactose, kg/d	1.41	1.63	1.49	1.61	1.57	0.15	0.004	0.82	0.34	0.06
MUN, mg/dL	14.37	13.56	14.18	13.20	13.86	0.49	0.09	0.96	0.94	0.03
SCC × 1,000/mL	205	205	175	216	221	113	1.00	0.18	0.24	0.13
3.5 % FCM/DMI	1.62	1.32	1.42	1.39	1.31	0.11	<0.001	0.82	0.09	0.72
ECM/DMI	1.64	1.32	1.41	1.39	1.31	0.11	<0.001	0.86	0.07	0.89
Milk/DMI	1.56	1.38	1.42	1.39	1.34	0.11	0.008	0.49	0.35	0.76

¹Dietary treatments were low starch diet (LS0, without autolyzed yeast (*Saccharomyces cerevisiae*) product), high starch diet (HS0, without yeast), 15 g (HS15), 30 g (HS30), and 45 g of yeast (HS45) in a top dress. Top dress vehicle was 300 g of ground corn. LS0 and HS0 treatments received 300g of corn in a top dress.

²Contrasts were LS0 compared with HS0; and the linear, quadratic, and cubic effects of treatments (HS0, HS15, HS30, and HS45).

³Greatest value within treatment standard error of the mean.

Table 2.4 Least squares means and associated standard errors for rumen pH, fecal pH, and VFA response in a low starch diet without autolyzed yeast product (LS0), high starch diet without autolyzed yeast product (HS0), HS with 15 g (HS15), 30 g (HS30), and 45 g (HS45) of autolyzed yeast

Variable	Treatment ¹					SEM	<i>P</i> -value Contrasts ²			
	LS0	HS0	HS15	HS30	HS45		LS0 vs. HS0	Linear TRT	Quad TRT	Cubic TRT
Rumen fluid										
pH ³	6.38	6.10	6.15	6.19	6.11	0.05	<0.001	0.67	0.04	0.45
pH <5.6, h ⁴	6.28	7.15	5.97	6.62	8.08	1.50	0.62	0.49	0.23	0.83
Nadir pH	5.74	5.53	5.55	5.57	5.57	0.03	<0.001	0.009	0.52	0.53
AUC, pH × h/d ⁵	0.07	0.26	0.12	0.15	0.11	0.12	0.23	0.25	0.60	0.55
Fecal pH	6.95	6.71	6.72	6.59	6.66	0.08	0.042	0.43	0.75	0.35
Total VFA, mmol/L	127.1	136.7	123.0	134.1	134.4 3	3.07	<0.001	0.75	0.06	0.09
Individual VFA, mol/100 mol total VFA										
Acetate ⁶	65.51	62.41	62.88	61.98	61.25	1.62	<0.001	<0.001	0.02	0.09
Propionate ⁷	20.75	23.87	23.74	24.63	24.79	2.60	<0.001	0.002	0.54	0.12
Butyrate	10.92	10.81	10.50	10.54	10.99	0.19	0.54	0.27	0.001	0.93
Isobutyrate	0.85	0.78	0.80	0.76	0.73	0.02	<0.001	<0.001	0.06	0.28
Valerate	1.35	1.50	1.47	1.51	1.50	0.07	<0.001	0.70	0.56	0.36
Isovalerate	0.63	0.60	0.60	0.58	0.57	0.02	0.02	0.01	0.31	0.88

¹Dietary treatments were low starch diet (LS0, without autolyzed yeast (*Saccharomyces cerevisiae*) product), high starch diet (HS0, without yeast), 15 g (HS15), 30 g (HS30), and 45 g of yeast (HS45) in a top dress. Top dress vehicle was 300 g of ground corn. LS0 and HS0 treatments received 300g of corn in a top dress.

²Contrasts were LS0 compared with HS0; and the linear, quadratic, and cubic effects of treatments (HS0, HS15, HS30, and HS45).

³Time point: $P < 0.0001$; treatment × time point: $P = 0.64$.

⁴During the first 24 h. Time points (TP) 0, 4, 8, 12, 16, 20, 24h relative to feeding at 1400 h.

⁵Negative incremental area under the curve. Baseline rumen pH = 5.6.

⁶Time: $P < 0.0001$; Treatment × time: $P < 0.01$ (Figure 2).

⁷Time: $P < 0.0001$; Treatment × time: $P < 0.0001$ (Figure 3). All other variables TRT × DAY interaction was not present ($P > 0.33$).

Table 2.5 Least squares means and associated SEM for blood metabolites of Holstein cows in low starch diet without autolyzed yeast product (LS0), high starch diet without autolyzed yeast product (HS0), HS with 15 g (HS15), 30 g (HS30), and 45 g (HS45) of autolyzed yeast during the experimental period. Samples were collected on d 15, 18, and 21 during the last week of each period

Variable	Treatment ¹					SEM	P-value Contrasts ²			
	LS0	HS0	HS15	HS30	HS45		LS0 vs. HS0	Linear TRT	Quad TRT	Cubic TRT
Blood ³										
Metabolism										
Glucose, mg/dL	70.15	70.14	71.90	71.53	70.52	1.03	1.00	0.79	0.03	0.61
GLDH ⁴ , U/L	32.67	31.13	32.10	32.69	32.78	5.50	0.48	0.46	0.78	0.99
Cholesterol total, mg/dL	171	174	171	173	178	14	0.45	0.29	0.19	0.92
BHB, mmol/L	0.58	0.52	0.52	0.48	0.47	0.03	0.12	0.08	0.77	0.58
Triglycerides, mg/dL	8.18	7.81	8.18	8.43	7.83	0.59	0.36	0.80	0.08	0.57
NEFA ⁵ , μ Eq/L	93.69	82.71	87.16	85.13	81.34	4.08	0.04	0.62	0.20	0.71
Insulin, μ g/L	0.74	0.93	0.86	0.95	0.92	0.09	0.03	0.84	0.76	0.31
Creatinine, mg/dL	0.66	0.65	0.65	0.64	0.65	0.02	0.23	0.79	0.66	0.65
CPK ⁶ , U/L	139.5	147.8	130.5	140.6	137.9	9.4	0.91	0.34	0.14	0.79
Anion Gap, mEq/L	17.44	17.07	17.53	17.09	16.60	0.29	0.19	0.03	0.02	0.33
Bicarbonate, mmol/L	26.90	26.80	26.13	26.69	26.80	0.36	0.83	0.68	0.19	0.21
D-Lactate, mM	0.57	0.57	0.63	0.57	0.60	0.03	0.97	0.98	0.55	0.07
Total protein, g/dL	7.60	7.43	7.66	7.64	7.56	0.42	0.03	0.37	0.05	0.49
Albumin, g/dL	3.38	3.34	3.39	3.33	3.30	0.04	0.27	0.08	0.11	0.30
Globulin, g/dL	4.17	4.09	4.24	4.31	4.22	0.44	0.23	0.16	0.03	0.79
Albumin/Globulin ratio	0.82	0.83	0.81	0.79	0.81	0.03	0.37	0.07	0.06	0.28
Minerals										
Calcium, mg/dL	9.31	9.12	9.29	9.34	9.20	0.09	0.07	0.37	0.03	0.82
Phosphorus, mg/dL	5.38	5.86	5.85	5.74	5.80	0.15	0.005	0.60	0.74	0.61
Sodium, mmol/L	136.55	136.20	136.77	136.60	135.50	0.55	0.68	0.03	0.07	0.56
Potassium, mmol/L	4.39	4.38	4.44	4.41	4.40	0.06	0.98	0.89	0.50	0.63
Na:K Ratio	31.13	31.11	30.96	31.07	30.80	0.42	0.98	0.59	0.87	0.68
Chloride, mmol/L	96.46	96.95	97.58	97.33	96.69	0.46	0.37	0.54	0.09	0.77
Magnesium, mg/dL	2.40	2.37	2.38	2.44	2.39	0.03	0.35	0.35	0.20	0.25
Liver function										
AST ⁷ , U/L	72.28	69.44	68.53	69.33	66.88	3.93	0.13	0.23	0.55	0.39
GGT ⁸ , U/L	24.83	26.08	25.70	25.20	25.64	1.25	0.02	0.26	0.25	0.51
Total bilirubin, mg/dL	0.14	0.12	0.12	0.11	0.12	0.009	0.05	0.63	0.43	0.08
Alkaline phosphate total, U/L	45.83	46.25	43.63	46.69	45.22	2.85	0.71	1.0	0.46	0.004
Inflammation										
Haptoglobin, μ g/mL	12	9	8	7	16	16	0.41	0.06	0.52	0.49
SAA ⁹ , μ g/mL	142	108	161	140	150	25	0.48	0.06	0.05	0.21
LBP ¹⁰ , μ g/mL	20.4	22.0	20.6	21.3	20.5	2.4	0.51	0.60	0.88	0.62
SOD ¹¹ , U/mL	3.67	4.09	3.77	3.59	3.50	0.32	0.19	0.05	0.61	0.96
GSH-Px ¹² , nmol/min/mL	87.0	84.0	87.7	81.5	88.8	4.2	0.55	0.60	0.60	0.13

¹Dietary treatments were low starch diet (LS0, without autolyzed yeast (*Saccharomyces cerevisiae*) product), high starch diet (HS0, without yeast), 15 g (HS15), 30 g (HS30), and 45 g of yeast (HS45) in a top dress. Top dress vehicle was 300 g of ground corn. LS0 and HS0 treatments received 300g of corn in a top dress.

²Contrasts were LS0 compared with HS0; and the linear, quadratic, and cubic effects of treatments (HS0, HS15, HS30, and HS45).

Day differed ($P < 0.05$) for potassium, Na:K ratio, chloride, BHB, AST, magnesium, anion gap, D-lactate, and GSH-Px. TRT $\times$ DAY interaction was not present for any variable ($P > 0.12$).

³All samples were run on blood plasma except insulin, and D-lactate.

Table 2.5 (cont.)

⁴Glutamate dehydrogenase.

⁵Non-esterified fatty acid.

⁶Creatine phosphokinase.

⁷Aspartate aminotransferase.

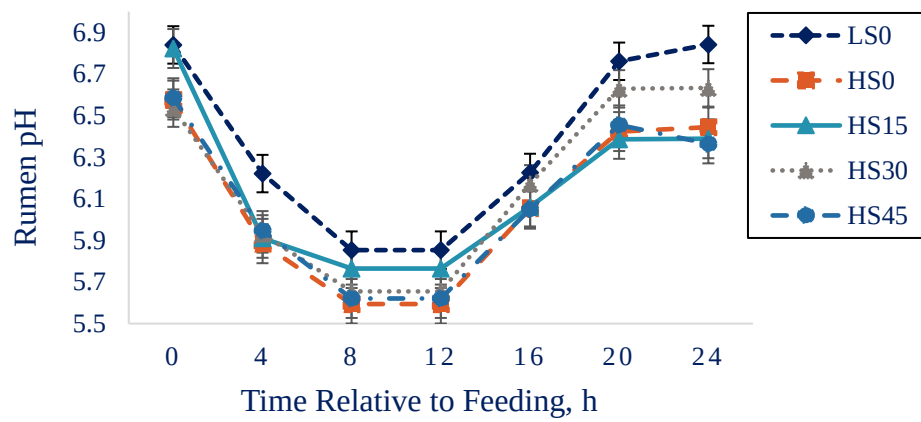
⁸Gamma-glutamyl transpeptidase

⁹Serum amyloid A.

¹⁰Lipopolysacchride binding protein.

¹¹Superoxide Dismutase. One unit (U) is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

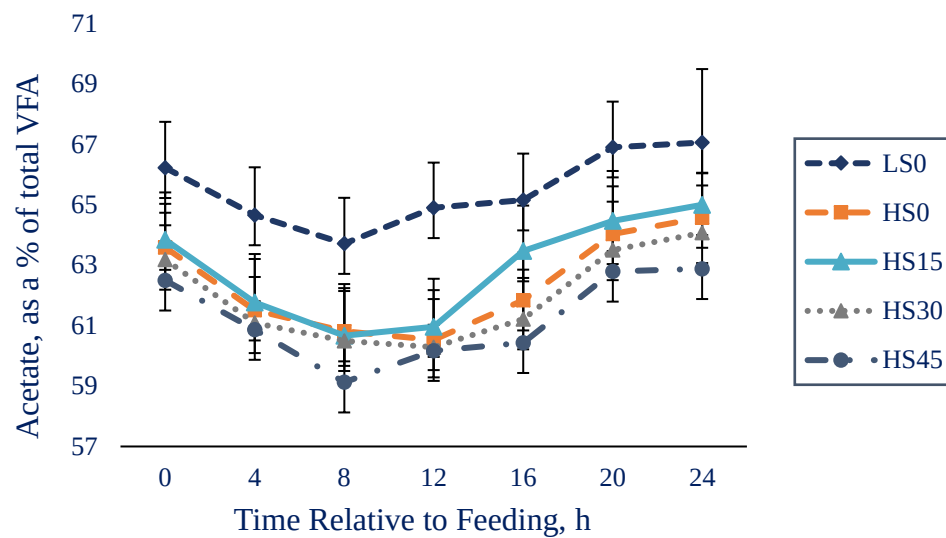
¹²Glutathione peroxidase activity. One unit (nmol/min) is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol of NADPH to NADP⁺ per minute at 25°C.



Knollinger et al., Figure 1

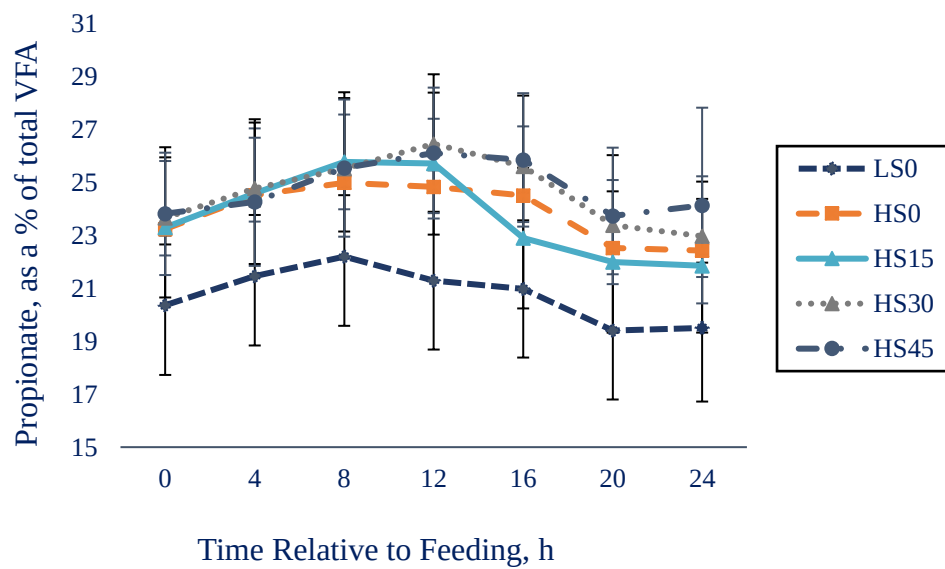
Figure 2.1 Least squares means ($\pm$ SE) for rumen pH response to feeding (0 h) for cows in LS0, HS0, HS15, HS30, and HS45 treatments from 0 to 24 h relative to feeding (at 1400 h).

Treatment: $P < 0.0001$; time: $P < 0.0001$; and no time $\times$ treatment interaction: $P = 0.64$.



Knollinger et al., Figure 2

Figure 2.2 Least squares means ($\pm$ SE) for acetate, as a % of total VFA response to feeding (0 h) for cows in LS0, HS0, HS15, HS30, and HS45 treatments from 0 to 24 h relative to feeding (at 1400 h). Treatment: $P < 0.0001$; time: $P < 0.0001$; and treatment $\times$ time interaction: $P = 0.0112$.



Knollinger et al., Figure 3

Figure 2.3 Least squares means ($\pm$ SE) for propionate, as a percentage of total VFA response for cows in LS0, HS0, HS15, HS30, and HS45 treatments from 0 to 24 h relative to feeding (at 1400 h). Treatment: $P < 0.0001$; time: $P < 0.0001$; and treatment $\times$ time interaction: $P < 0.0001$.

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CHAPTER III

AUTOLYZED YEAST SUPPLEMENTATION REDUCES FECAL NITROGEN EXCRETION, IMPROVES DIGESTIBILITY, AND IN SITU RATE OF DEGRADATION WHEN FED TO DAIRY COWS

ABSTRACT

Diets containing excess N increase fecal N excretion and can have negative impacts on the environment and animal production. The aim of this study was to investigate the opportunity improve N utilization, nutrient bioavailability, apparent digestibility, and in situ degradability when supplementing autolyzed yeast (**AY**). Fifteen rumen-cannulated Holstein cows were assigned to 1 of 5 treatments in a replicated 5 × 5 Latin square design balanced to measure carryover effects. Treatments were: low starch diet without AY (**LS0**, control), high starch diet without AY (**HS0**), high starch diet with either 15 g (**HS15**), 30 g (**HS30**), or 45 g (**HS45**) of AY supplementation. Periods (21 d) were divided into the adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). During the measurement phase, total mixed ration samples were collected on d 18 through 20 and orts on d 18 through 21 and analyzed for dry matter, neutral detergent fiber (NDF), crude protein, starch, organic matter (OM), total N, and undegradable NDF (**uNDF**) 120 h. Urine samples were collected at 0800, and 2030 h on d 20 of each period. Fecal samples were collected on d 18 through 20. In situ degradability determination of alfalfa hay, corn silage (**CS**), and soybean meal was performed on d 19 through 21 of each period. One single degree-of-freedom contrast LS0 compared to HS0 was used, and linear, quadratic, and cubic treatment effects of HS0, HS15, HS30, and HS45. Cows in HS0 had greater N intake (719.6 vs. 583.7 g/d), milk protein N (166.0 vs. 140.3 g/d), and fecal N (268.4 vs. 219.8 g/d) with a tendency for greater microbial N production when compared to LS0. Supplementing AY decreased fecal N excretion, lowest at HS15. Cows in HS0 had greater nutrient intakes for OM

(22.6 vs. 18.6 kg/d), crude protein (4.5 vs. 3.7 kg/d), and starch (6.6 vs. 4.7 kg/d) when compared to cows in LS0. Cows in LS0 had a tendency for greater digestibility of starch (95.0 vs. 93.7 %) and NDF (52.0 vs. 45.7 %), when compared to HS0, AY increased starch and NDF digestibility. Cows in LS0 had a greater degradable fraction of OM (0.35 vs. 0.28), NDF (0.75 vs. 0.37), and acid detergent fiber (0.66 vs. 0.43) for CS when compared to HS0. For CS NDF k_d and acid detergent fiber degradable fraction tended to increase with AY, while undegradable starch decreased. In conclusion, cows in HS0 produced and excreted more N compared to LS0 and the addition of AY improved fecal N excretion, rate of degradation, and degradability.

Keywords: nitrogen, digestibility, degradability, yeast, high starch

INTRODUCTION

Increased environmental regulations have created new pressures for nutrient use improvements for the agricultural industry (Powell et al., 2010). The release of N in the environment can negatively affect human health through the combination of ammonia and other chemicals in the atmosphere (Powell et al., 2010). Therefore, the excretion of nitrogen is becoming more of a concern on commercial dairy farms. Fecal and urinary nitrogen, through bacterial degradation can be converted to ammonia. The Environmental Protection Agency (EPA) classifies ammonia as an air quality threat because it can contribute to nitrate contamination of groundwater, surface water eutrophication, and hinder air quality (EPA, 2019). Dietary factors in the diet such as CP concentration, in addition to carbohydrate and forage type, can have an impact on the quantity and form of N excretion, whether fecal or urinary (Weiss et al., 2009). In addition, increases in protein supplementation of dairy cows can increase N pollution. High-producing dairy cows require sufficient energy-dense diets to meet their needs for lactation. Typically, peak lactation diets are approximately 17 to 18% CP on a

DM basis and high in starch content. Diet manipulations may aid in maximizing N utilization and thus, reduce N pollution by dairy cows (Tamminga, 1992). Increasing milk yield can reduce the proportion of energy used for maintenance (Tamminga, 1992). According to Broderick (2003) and Rius et al. (2010), the increased consumption of nonstructural carbohydrate, predominantly corn starch, increases milk yield, milk protein yield, and thus, N efficiency of lactating dairy cows. Other ways to help minimize N excretion are the use of high quality feeds, reducing N intake, and reducing P in relation to energy. These practices will also help reduce CH₄ and production loss of endogenous protein, in addition to reducing fecal and urinary N excretion (Tamminga, 1992).

Direct-fed microbials, such as yeast, can help stabilize rumen fermentation by increasing beneficial bacteria, ruminal pH, and oxygen removal (Newbold et al., 1996; Chaucheyras-Durand et al., 2008). Rumen stabilization may improve degradability of feed and thus, increase nutrient utilization. In addition the inclusion of feed additives, such as yeast, has been shown to improve animal productivity via increased performance and digestion efficiency. Improved digestion efficiency increases the conversion of gross energy to digestible energy. Increasing the nutrients and/or energy digested can improve performance by increasing milk production and profit for the dairy producer. Yeast supplementation may interact with the N concentration in the diet (Moloney and Drennan, 1994). According to Julien et al. (2015), yeast supplementation, in particular, the *Saccharomyces cerevisiae* strain, may reduce N excreted in the feces and CH₄ production based on microbiome changes in the rumen (Lascano et al., 2012; Dias et al., 2018; Jin et al., 2017). Moreover, in vitro research has suggested yeast culture (YC) supplementation can increase the utilization of H₂ by acetogens, thus decreasing CH₄ production (Chaucheyras et al., 1995).

The objectives of this study were to investigate the opportunity to improve N utilization, nutrient bioavailability, apparent digestibility, and in situ degradability when supplementing autolyzed yeast (**AY**). This study postulated that AY supplementation would elicit changes in the rumen that would improve the efficiency of microbial N use, apparent digestibility, and ruminal degradability.

MATERIALS AND METHODS

Animal Care and Housing

All experimental procedures were approved by the University of Illinois (Urbana-Champaign) Institutional Animal Care and Use Committee (#17172). The experimental period was from January through April 2018. Cows were housed in tie stalls with sand bedding, fed *ad libitum*, and had unlimited water access. Diet (TMR) was formulated to meet or exceed NRC (2001) recommendations for cows at 70 DIM, 703 kg of BW, producing 41 kg of milk/d with a target 3.8% milk fat and 3.2% milk protein, and a predicted DMI of 25 kg/d (Knollinger et al., 2019, *submitted*).

Experimental Design

A total of 15 multiparous rumen-cannulated Holstein cows at the start of the experiment [BW (mean $\pm$ SD) = 623 $\pm$ 73 kg; DIM = 77 $\pm$ 26] were assigned to 1 of 5 treatments in a replicated 5 $\times$ 5 Latin square design balanced to measure carryover effects. Therefore, treatments were arranged so that the carryover effects could be evaluated. Periods (21 d) were divided into an adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). On d 1 to 21 of each period cows received 1 of 5 dietary treatments: low starch (**LS**) diet without autolyzed yeast (**LS0**) or high starch (**HS**) diet without AY (**HS0**), 15 g of AY (**HS15**), 30 g of AY (**HS30**), or 45 g of AY (**HS45**). The AY (*Saccharomyces cerevisiae*) product was spray-dried and produced

by an internal process technology for standardized autolytic degradation of the yeast cell (Levabon Rumen E, BIOMIN Holding GmbH, Austria). All cows were fed their respective diets once daily at 1400 h throughout the trial. The daily AY allocation was mixed with 300 g of ground corn and top-dressed onto the TMR immediately after feeding. Cows in LS0 and HS0 were given a top dress consisting of 300 g of ground corn only.

Data Collection and Sampling Procedures

Diet ingredient composition and nutrient analysis are explained elsewhere, in addition to production parameters (Knollinger et al., 2019, *submitted*). Total mixed ration offered and refused from each cow was recorded daily to determine intake based on weekly DM analyses. During the measurement phase, TMR samples were collected to determine apparent digestibility on d 18, 19, and 20, along with refusal collections on d 18, 19, 20, and 21 of each period for each individual cow and stored at -20°C until analyzed for DM, NDF, CP, starch, ash, total N, and uNDF 120 h as described by Farmer et al. (2014).

Urine samples (15 mL) were collected by manually stimulating urination at 0800 and 2030 h on d 20 of each period. Urine samples were immediately acidified after collection by diluting 1 volume of urine with 4 volumes of 0.072 N H_2SO_4 and stored at -20°C . Urine samples were later thawed and composited by volume by cow and period and analyzed for creatinine, uric acid, and urea N (Veterinary Medical Diagnostic Laboratory, MO), allantoin (Chen, 1989), and total N (Dairy One, Ithaca, NY). Daily urinary volume and excretion of total N, urea N, and allantoin were approximated from urinary creatinine concentration using a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999). Fecal samples (120 mL, wet weight) were collected directly from the cow's rectum on d 18, 19, and 20. On d 18 and 20 fecal pH was recorded (AP115, Fisher Scientific, Pittsburgh, PA). Fecal samples were

composited on an equal wet weight basis. Similar to Farmer et al. (2014), uNDF was used as an internal marker and apparent digestibility calculations were conducted by the ratio technique using the nutrient and indigestible NDF concentrations in the TMR and feces (Maynard et al., 1979) adjusted for each cow based on the nutrient composition of the diet offered and refused.

In situ degradability determination of a standard alfalfa hay (**AH**), corn silage (**CS**), and soybean meal (**SBM**) was performed using rumen-cannulated cows (n = 15), on d 19, 20 and 21 of each period. Each of the three ingredients were sampled from multiple location along the face of the bunker silo in order to retrieve a representative sample (Haerr et al., 2016). Nutrient chemical analysis for AH, CS, and SBM were analyzed for DM, CP, starch, ADF, and NDF (Table 1). Corn silage, AH, and SBM samples were dried in a forced air oven at 60°C (48, 24, and 24 h of dry time, respectively) and ground (Thomas-Wiley Laboratory Mill, Swedesboro, NJ) with a 4-mm screen (AH and CS) and 1-mm screen (SBM). The procedure used was comparable to the one described by Vanzant et al. (1998). All Dacron bags (Ankom Technology, Macedon, NY, USA), 10 × 20 cm with a 50 ± 10 µm porosity, were labeled, dried and weighed prior to the addition of AH, CS, and SBM. Furthermore, all bags were heat sealed at least twice to ensure no feed particles were lost. All bags were filled with 8 g of DM to attain 20 mg of DM/cm² of bag surface. All individual bag weights were recorded before and after adding 1 of the 3 designated ingredients. Three replicates were used for each ingredient (n = 3) at each time point (n = 5) for a total of 45 bags per treatment per cow. All bags were organized by cow, ingredient, and time point. There were 9 bags per 1 large 14" × 18" mesh garment bag. Color coordinated string was attached to each garment bags to aid in identification and prevent loss of bags in the rumen. Two washers (92g) were placed in each garment bag to help them sit lower in the rumen. All bags were saturated in warm water (45°C) for 30 minutes before placing them in

the rumen (Nocek 1988; Vanzant et al., 1998). The control bags (time point zero) were removed from warm water and placed in the freezer until further analyzed. All other bags were placed in the rumen and removed at a corresponding time point (4, 12, 24, or 48 h) keeping record of removal time. Air exposure was carefully minimized to avoid interference with routine fermentation. Once bags were removed from the rumen they were immediately placed in ice water buckets to stop fermentation. Bags were hand rinsed in cold running water and lightly squeezed until the water runoff was clear, then bags were immediately frozen at -20°C for a minimum of 24 h. Bags were then completely thawed and put in the washing machine (Roper RTW4641BQ1, Whirlpool Corp., Benton Harbor, MI, USA) on a $2 \times$ rinse cycle for removal of microbial debris. Bags were then immediately oven dried (AOAC, 1995) for 24 h at 60°C and disappearance was calculated. All bags were composited by cow, time point, and ingredient and sent to a commercial laboratory (Dairy One, Ithaca, NY) to be analyzed for the determination of nutrient disappearance (DM, OM, NDF, ADF, CP, and starch; Dairy One, Ithaca, NY).

Statistical Analyses

Data collected from d 15 to 21 of each period was analyzed using SAS (v. 9.4, SAS Institute Inc., Cary NC). For variables the MIXED procedure of SAS was used to model the fixed effects of treatment, square, and period:

$$Y_{ijk} = \mu + T_i + S_j + P_k + \varepsilon_{ijk}$$

where Y_{ijk} = the observations for dependent variables; μ = the overall mean; T_i = the fixed effect of the i^{th} treatment; S_j = effect of the j^{th} square; P_k = effect of the k^{th} period; and ε_{ijk} = the random residual error.

In situ degradability data was analyzed in two sequential steps. First, a nonlinear model of in situ digestion was analyzed using the NLREG procedure (Sherrod 1998), based on the

partitioning of feed such that the fractions of soluble feed (A), washout fraction after rinsing at time point 0), rumen degradable feed (B), and undegradable feed (C) is summed to 1. The disappearance data from the bags were first fitted to the full model:

$$Y = B (exp^{-V k_d(t-t_1)}) + C$$

where B = the proportion of potentially degradable feed, C = the proportion of undegradable feed, t = time point, Y = the proportion of nutrient remaining at a specified time point (t), k_d = the fractional digestion rate constant, and V = 1 when $t \geq \text{lag } (t_1)$, and V = 0 when $t < \text{lag}$ (McDonald, 1981; Van Milgen et al., 1991). The parameter of lag was restricted so that $\text{lag} \geq 0$. If the full model failed to converge or $\text{lag} = 0$ then the reduced model was utilized (Ørskov and McDonald, 1979):

$$Y = B (exp^{-k_d(t)}) + C$$

The A (soluble) fraction was then calculated as $1 - (B + C)$. The effective degradability (**ED**) was estimated as $ED = A + B \times [k_d / (k_d + k_p)]$ (McDonald, 1981). Although, the fractional rate of passage from the rumen (k_p) is affected by many factors [feeding intake, diet composition, feed particle size, and moisture in feeds (Krizsan et al., 2010)], a value of 0.06 was assumed to estimate ED in the present experiment. An ANOVA was then conducted on estimates of soluble feed, rumen degradable feed, undegradable feed, k_d , and ED. Secondly, a linear mixed model (MIXED procedure of SAS) was used to model the fixed effects of treatment, square, and period. For all models, cow was the experimental unit and considered as a random effect. The experimental design was balanced to measure carryover effects. One single degree-of-freedom contrast was used LS0 compared with HS0, along with the linear, quadratic, and cubic treatment effects for HS0, HS15, HS30, and HS45. Values reported are least squares means and associated standard errors of the mean. Degrees of freedom method was Kenward-Rogers (Littell, 2002). Residuals

distribution was evaluated for normality and homoscedasticity. Statistical significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Carryover effect was tested and was not present for any variable of interest ($P > 0.31$).

Production data are described in Knollinger et al., 2019, *submitted*. Briefly, cows in HS0 had increased DMI (24.9 kg/d), BW (689 kg), and milk yield (34.1 kg/d) compared to LS0 (19.9 kg/d, 665 kg, and 30.9 kg/d, respectively; $P < 0.001$, $P = 0.003$, $P = 0.02$, respectively). Cows in HS0 had lower ruminal pH (6.10), nadir pH (5.53), and fecal pH (6.71) when compared to LS0 (6.38, 5.74, and 6.95, respectively; $P < 0.001$, $P < 0.001$, $P = 0.042$, respectively).

Nitrogen Excretion and Apparent Digestibility

Nitrogen excretion and apparent digestibility data for the measurement phase are in Table 2. We observed no linear treatment effects for N intake, milk protein, PUN, or urinary excretion parameters. Cows in HS0 had greater N intake ($P = 0.005$), milk protein N yield ($P = 0.038$), and a tendency for greater urea N, as a percentage of total urinary N ($P = 0.08$) when compared to LS0. A tendency for a cubic treatment effect was present for N intake ($P = 0.07$), and a difference for milk protein N ($P = 0.05$), and total N, as a percentage of N intake ($P = 0.04$), and PUN ($P = 0.004$). A tendency for a quadratic treatment effect was present for urine allantoin ($P = 0.09$). We observed a quadratic treatment effect present for uric acid ($P = 0.03$). Cows in HS0 tended to have greater total purine derivatives (**PD**; $P = 0.09$) and microbial N production ($P = 0.09$) when compared to LS0. Cows in HS0 had greater fecal N excretion ($P = 0.01$) compared to LS0. We observed a decrease in fecal N excretion ($P = 0.03$) with AY supplementation, and a tendency for a linear treatment effect for fecal N, as a percentage of intake ($P = 0.10$). Cows in HS0 had greater nutrient intakes for OM ($P = 0.0006$), CP ($P = 0.003$), and starch ($P < 0.0001$)

when compared to LS0. A tendency for a quadratic treatment effect was present for OM ($P = 0.08$) and NDF ($P = 0.09$) nutrient intakes. A tendency for a cubic treatment effect was present for CP ($P = 0.10$) and starch ($P = 0.07$) nutrient intakes. A positive linear tendency was present for apparent digestibility of CP ($P = 0.07$), in addition to cubic treatment effects for starch ($P = 0.02$) and NDF ($P = 0.05$) digestibility with AY. Cows in LS0 tended to have greater apparent digestibility of starch ($P = 0.08$) and NDF ($P = 0.08$) compared to HS0.

In Situ Degradability

Alfalfa hay, CS, and SBM in situ degradability data for the measurement phase are in Table 3, 4, and 5, respectively. We observed no AH treatment differences for LS0 versus HS0, or cubic treatment effects. We observed a linear treatment effect for undegradable OM ($P = 0.02$) and NDF ($P = 0.05$) for AH. Both a positive linear ($P = 0.08$) and quadratic ($P = 0.08$) tendency was present for rate of ADF degradation (k_d) for AH. We observed no linear or quadratic treatment effects for CS. Cows in HS0 tended to have greater soluble OM ($P = 0.10$) for CS when compared to LS0. Cows in LS0 had greater OM ($P = 0.05$), NDF ($P = 0.007$), and ADF ($P = 0.04$) degradability for CS when compared to HS0. Cows in HS0 had more undegradable NDF ($P = 0.009$), and ADF ($P = 0.02$) for CS when compared to LS0. Tendencies for cubic treatment effects were present for CS rate of NDF degradation ($P = 0.10$), ADF degradable ($P = 0.08$), and undegradable fractions ($P = 0.06$). A tendency for a cubic treatment effect was present for CS starch undegradable fraction ($P = 0.08$). Cows in LS0 tended to have a greater fractional rate of OM degradation ($P = 0.07$) for SBM when compared to HS0. We observed a quadratic tendency for OM degradable fraction ($P = 0.09$) and a positive linear tendency for rate of OM degradation ($P = 0.07$) for SBM. Cows in LS0 had a greater CP undegradable fraction ($P = 0.001$), and rate of CP degradation ($P = 0.03$) for SBM when compared to HS0. Cows in LS0 tended to have greater ED for CP ($P = 0.08$) for SBM when compared to HS0.

DISCUSSION

In this study, we postulated that AY supplementation would elicit changes in the rumen that would improve the efficiency of microbial N use, apparent digestibility, and ruminal degradability. All diet treatments were isonitrogenous (17.5 ± 0.56 % CP, as a percentage of DM) however, cows in HS0 had greater N intake when compared to LS0. The greater N intake was because cows in HS0 consumed greater DMI, also resulting in increased milk protein N when compared to the LS treatment (Knollinger et al., 2019, *submitted*). Similarly, increased N intake and milk protein N was observed with increased AY and DMI. Aguerre et al. (2011) reported 93 % of N intake by cows was in fecal N, milk N, or emitted as $\text{NH}_3\text{-N}$. Moorby et al. (2006) reported increased N intake, milk N, fecal N, and DMI with greater concentrate inclusion, similar to the current study. However, Dias et al. (2018) reported no differences in N intake, and increased milk N for HS (29.0 ± 1.2 % starch DM) versus LS (23.2 ± 1.7 % starch DM) treatments and increased milk N with yeast supplementation but no difference for DMI across treatments. Thus, increased milk N reported in the current study for HS diets may not just be a result of greater intakes but changes in the microbial protein yield eliciting changes in amino acid profile in the small intestine between the two diets (Dias et al., 2018).

Cows consuming a HS diet had greater CP nutrient intakes, which can increase RDP into microbial protein, thus increasing N utilization efficiency (Sommerfeldt and Lyon, 1988; Knollinger et al., 2019, *submitted*). Cows in HS0 tended to have greater total purine derivatives (PD) and microbial N production when compared to the LS0 treatment. The reason greater concentrate diets may increase microbial protein is because additional energy substrate provided to ruminal microorganisms may improve the efficiency of fermentation in the rumen, thus enhancing the capture of ruminal ammonia into microbial protein (Agle et al., 2010). Likewise,

Valadares et al. (1999) reported that total PD and microbial N increases as the concentrate to forage ratio (**C:F ratio**) increases (20 to 65 dietary concentrate, % of DM).

Agle et al. (2010) reported no treatment differences for urine allantoin, uric acid, total PD, or microbial protein when feeding high concentrate (29.6 % starch DM) versus low concentrate (21.3 % starch DM). However, the addition of AY tended to decrease urine allantoin and increased uric acid, but contrary to the current study Hristov et al. (2010) reported a tendency for increased urine allantoin with yeast supplementation with no difference for uric acid. The LS0 treatment, assuming greater prevalence in rumen fibrolytic bacteria compared to HS0, could aid in improved efficiency of conversion of ruminal ammonia to microbial N production (Hristov et al., 2010); therefore, this may help explain the lower urea N as a percentage of total urinary N tendency, as urinary N is the main source of ammonia excretion from cattle. This could decrease ammonia emissions from feces (Hristov et al., 2010), as reported by the reduction in fecal N excretion by cows in LS0. Urine N efficiency (Total N, as a percentage of N intake), was the most efficient at HS30, as well as had improved MUN and plasma urea nitrogen (**PUN**). However, the HS30 dosage had the greatest fecal N excretion with AY (Knollinger et al., 2019, *submitted*).

Feeding HS diets have reported greater fecal N excretion when compared to LS diets, concurrent with the present study (Dias et al., 2018). Greater fecal N excretion in the HS diet may be correlated to the increased amount of N intake (Weiss et al., 2009; Dias et al., 2018). However, fecal N excretion decreases with YC compared to no yeast supplementation (Lascano et al., 2012; Dias et al., 2018), in the current study, fecal N excretion decreased by 10% with AY, the lowest N excretion at HS15. Yeast supplementation may modulate changes in the rumen, thus increasing fibrolytic bacteria that have a high inclination for ammonia as an N source

(Bryant, 1973) if this is the case increased utilization of ammonia in the rumen for synthesis of microbial protein may be expected, improving overall dietary N efficiency (Hristov et al., 2010). A decrease in fecal N excretion by AY supplementation can have a positive impact on the environment (Powell et al., 2010; Hernandez et al., 2016).

Nutrient intakes and apparent digestibility for cows fed HS and LS diets in the current study are similar to Lascano et al. (2012), which reported that feeding HS (27.9 % starch DM) had greater OM, and starch nutrient intakes when compared to feeding LS (16.7 % starch DM). However, Lascano et al. (2012) reported no linear or quadratic treatment differences for YC dose on nutrient intakes. However, with increased DMI at HS30 and HS45 increased nutrient intakes for CP and starch were observed with AY. In addition, similar to the present study, the apparent digestibility for OM was not different between the HS and LS treatments (Lascano et al., 2012). Many studies have reported improved OM digestibility when *S. cerevisiae* was supplemented because of increased fiber digestion (Desnoyers et al., 2009; Lascano et al., 2012).

Contrary to the current study, a study with treatments varying in F:C ratio (80:20, 65:35, 50:50, and 35:65 % of DM) on apparent digestibility of OM, N, and starch were all unaffected by treatment (Moorby et al., 2006). However, Mwenya et al. (2005) reported that cows supplemented with YC had numerically greater CP digestibility (74.5 vs. 75.9 % CP), and Wohlt et al. (1991) reported a CP digestibility tendency (73.8 vs. 75.4 % CP) with yeast supplementation concurrent with the current study. Additionally, previous studies (Erasmus et al., 1992; Dias et al., 2018) reported significant increases in CP digestibility with YC supplementation. Moorby et al. (2006) observed the amount of dietary N digested in the rumen increased with greater feed intake. Cows in the HS45 treatment had the greatest DMI along with the greatest apparent CP digestibility (Knollinger et al., 2019, *submitted*).

A study conducted by Farmer et al. (2014) observed decreasing the forage in the diet reduced the NDF apparent digestibility, consistent results with the current study (Yang et al., 2001; Beckman and Weiss, 2005; Moorby et al., 2006). Agle et al. (2010), in agreement with the present study, reported lower apparent NDF digestibility in higher concentrate (29.6% starch in DM) diets when compared to lower concentrate (21.3% starch in DM). Increasing the amount of rapidly fermentable starch in the diet, while decreasing forage fiber can increase VFA production beyond buffering and absorptive ability thus reducing ruminal pH, which can have negative implications on fiber digestion, as observed in the present study (Hatew et al., 2015; Knollinger et al., 2019, *submitted*). However, with AY supplementation NDF and starch digestibility improved, greatest at HS15. In addition, starch digestibility tended to decrease in the HS0 diet compared to LS0; previous reports observed a positive linear increase in starch whole-tract digestibility with increased concentrate (Moorby et al., 2006). According to Firkins et al. (2001), on average, for every one-percentage unit increase of dietary NDF concentration approximately 0.6 percentage units of starch digestibility decreases. Prior studies have reported no starch treatment differences for high (32.9 % starch in DM) vs. low concentrate (24.1 % starch in DM; Yang et al., 2001). It is reported that approximately 90 % or more of starch is fermented in the rumen for most grains (Ørskov and McDonald, 1979). The decreased rumen pH observed when feeding the HS0 treatments may explain the decrease in starch digestibility when compared to the LS0 treatment (Knollinger et al., 2019, *submitted*). Subsequently, the enzyme activity of rumen fluid could affect starch digestibility (Oba and Allen, 2003). Furthermore, in the LS0 treatment, a greater forage DM percentage may have increased substrate availability for ruminal microbes compared to HS0 thus, improving digestibility.

The difference between feeding cows LS or HS diets have diverse impacts on the rumen environment, thus the effect of degradability for commonly fed feedstuffs, AH, CS, and SBM may affect the nutritive capability and nutrient utilization by dairy cows (Nocek, 1988). It is important to keep in mind the particle size (4-mm screen for AH and CS; 1-mm screen for SBM) may influence the rate of passage and rate of degradability (Galyean et al., 1979). In particular, smaller particle sizes will pass faster through the rumen, which can cause decreased degradability (NRC, 2001). Furthermore, diet composition, in particular varying starch content, effects on ruminal microbial population may potentially affect the extent of degradability, rate of degradability, or both (Vanzant et al., 1998).

In the current study, CS was more degradable for the LS0 treatment; thus, the increase in OM, NDF, and ADF degradability for LS0 could be a result of higher ruminal pH and more microbial metabolism. Furthermore, SBM in the LS0 treatment had a faster fractional rate of DM degradation. This may be explained by the fact that feeding high-starch diets are prevalent in *Streptococcus bovis* bacteria that are known to produce slime that could potentially block the pores on the bags (Galyean and Rivera, 2003). However, the bags may not have been in the rumen long enough for this to occur (24 h). Additionally, a more favorable rumen environment (increased pH, and saliva production), the forage mat in the rumen, and abrasive action during fermentation may aid in clearing foreign material from pores thus increasing degradation of available substrates (Vanzant et al., 1998).

The differences observed between the two feed ingredients CS and SBM could be because CS is more forage-based with different nutrient composition, and larger in particle size compared to SBM, thus CS had a lower degradable fraction value. Yeast supplementation is known to positively affect the microbial population; in particular, increase cellulolytic bacteria

while alleviating negative associations from high concentrate diets (Shurson 2018). However, in the present study, such effects were not evident in AH as the OM and NDF undegradable fraction increased with AY. Furthermore, this did not occur for the other ingredients CS and SBM suggesting AH and AY supplementation may influence the rumen microbes differently than AY supplementation with CS or SBM. Interestingly, the OM and NDF undegradable fraction values, when supplemented with AY, more closely numerically mimic the LS0 diet for AH.

When feeding the LS0 treatment, the OM, NDF, and ADF degradable fractions for CS were greater compared to HS0; this could be because the LS0 has a greater fluid ratio volume, due to the additional saliva produced from forage substrates. A slower passage rate for the LS0 diet due to increased forage in the diet could allow for greater degradation (Nocek, 1988). In addition to the increased benefit from saliva production, this could explain why the ADF rate of degradation fraction is greater for the LS0 treatment and tended to increase with AY supplementation. The LS0 diet for CS had lower undegradable fractions and with the addition of AY the undegradable starch and ADF fractions in CS decreased, lowest at HS15; however, the values are very minimal.

Crude protein degradability for SBM in the LS0 diet had a greater undegradable fraction portion, this may suggest less ammonia in the rumen and lower urinary N excretion. Nocek et al. (1988) reported degradation of N components are protected by structure found in fibrous feeds; however, the type of forage is a factor to consider. Therefore, when feeding HS diets and depressed fiber digestibility is apparent, as observed in the current study, this could imply the depression of N degradation for the HS0 diet. Moreover, the fractional rumen outflow rate could negatively impact dietary protein degradability (Wiedmeier et al., 1987), decreasing the time

available for fermentation. In addition, cows in LS0 had a greater rate of degradation and a tendency for effective degradability. Previous studies have reported in situ forage degradability for animals consuming concentrates (HS0) decreases compared to animals on a forage based diet (LS0; Lindberg, 1981; Cronje, 1992).

Overall, the HS0 treatment had more undegradable fractions for CS when compared to the LS0 diet. This could be in part to the fractional passage rate and overall rumen environment characteristics, lower ruminal pH, and greater VFA production when feeding a HS0 diet (Knollinger et al., 2019, *submitted*). Feeding the LS0 diet allowed for a greater degradable fraction of OM, NDF, and ADF in CS when compared to the HS0 treatment. The addition of AY closely mimicked LS0 diet for in situ degradability. The rumen microbiome, influenced by diet, and its effect on digestion and in situ degradability would be interesting to investigate

CONCLUSION

In conclusion, the results from the present study indicate that feeding HS increased N intake, and tended to increase microbial N production with greater excretion of fecal N and secretion of milk N protein compared to LS diets. Additionally, feeding high concentrate diets increased OM, CP, and starch nutrient intakes while decreasing the starch and NDF apparent digestibility in comparison to feeding more forage based diets. However, the addition of AY reduced fecal N excretion, and increased CP, starch, and NDF apparent digestibility, which may positively impact environmental concerns arising due to excessive excretion of N by dairy cows. In situ degradability for HS diets had greater undegradable fractions and a slower rate of degradation for SBM when compared to LS. Supplementation of AY tends to improve corn starch degradation, and increases AH ADF and SBM OM rate of degradation in situ. We observed the best AY dosage for improvements on digestibility, N utilization, and ruminal

degradability is at the lower AY concentrations (HS15, and HS30). Further research investigating the rumen microbiome may help explain the role of bacteria in the rumen and its effect on digestibility and in situ degradability.

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TABLES

Table 3.1 Mean chemical composition of ingredients used to determine degradability in Holstein cows in a low starch diet without autolyzed yeast (AY) product (LS0), high starch diet without AY product (HS0), HS with 15 g (HS15), 30 g (HS30), and 45 g (HS45) of AY

	Mean
Alfalfa hay	
DM	88.8
CP	19.5
Starch	1.7
ADF	37.7
NDF	49.5
Corn silage	
DM	29.4
CP	9.5
Starch	32.5
ADF	22.8
NDF	37.0
Soybean meal	
DM	90.1
CP	52.3
Starch	1.2
ADF	6.6
NDF	8.5

Abbreviations: acid detergent fiber (ADF); crude protein (CP); dry matter (DM); neutral detergent fiber (NDF)

Table 3.2 Least squares means and associated SEM of nitrogen excretion and apparent digestibility in low starch diet without autolyzed yeast product (LS0), high starch diet without autolyzed yeast product (HS0), HS with 15 g (HS15) , 30 g (HS30), and 45 g (HS45) of autolyzed yeast during the experimental period

Variable	Treatment ¹					SEM	P-value Contrasts ²			
	LS0	HS0	HS15	HS30	HS45		LS0 vs. HS0	Linear TRT	Quad TRT	Cubic TRT
N intake, g/d	613	719	652	730	738	43	0.005	0.22	0.15	0.07
Milk protein N ³ , g/d	140.29	165.99	149.65	176.40	170.45	16.61	0.038	0.28	0.53	0.05
Milk protein N, % of N intake	22.82	22.60	22.15	24.27	22.75	1.96	0.88	0.58	0.61	0.19
Urinary excretion										
Urine volume ⁴ , L/d	39.87	37.23	38.01	36.65	40.94	3.47	0.53	0.45	0.55	0.55
Total N, g/d	247.76	260.29	250.26	245.08	270.49	16.60	0.53	0.68	0.20	0.68
Total N, % of N intake	41.33	35.10	42.30	34.54	38.45	3.51	0.14	0.86	0.56	0.04
Urea N, g/d	200.99	215.37	212.04	213.30	227.22	12.32	0.30	0.39	0.37	0.85
Urea N, % of total urinary N	76.92	82.41	81.87	80.62	83.62	2.31	0.08	0.84	0.36	0.53
Allantoin, mmol/d	172.97	194.72	170.21	181.85	189.30	12.82	0.11	0.91	0.09	0.34
Uric acid, mmol/d	65.30	73.68	60.46	73.61	78.91	6.41	0.16	0.11	0.03	0.06
Total PD, mmol/d	220.61	255.07	225.43	240.16	245.12	21.56	0.09	0.81	0.22	0.39
Microbial N production ⁵ , g/d	138.85	160.53	141.88	151.15	154.27	13.57	0.09	0.81	0.22	0.39
PUN, mg/dL	14.79	14.30	15.75	13.93	15.27	0.62	0.47	0.61	0.90	0.004
Fecal N excretion										
N, g/d	219.78	268.36	225.84	260.48	242.38	20.28	0.01	0.46	0.36	0.03
N, % of intake	37.70	37.30	34.88	35.30	33.11	2.07	0.87	0.10	0.94	0.46
Nutrient intakes, kg/d										
OM	18.55	22.57	20.55	22.20	22.87	1.27	<0.001	0.45	0.08	0.18
CP	3.65	4.50	4.08	4.59	4.61	0.30	0.003	0.31	0.25	0.10
Starch	4.67	6.56	5.98	6.95	6.94	0.45	<0.001	0.12	0.36	0.07
NDF	6.15	6.53	6.03	6.16	6.45	0.36	0.26	0.90	0.10	0.66
Apparent digestibility, %										
OM	65.46	65.12	67.66	65.95	68.19	1.33	0.85	0.16	0.90	0.13
CP	61.06	61.75	64.05	63.33	66.07	1.90	0.75	0.07	0.88	0.34
Starch	95.05	93.71	95.17	93.72	94.72	0.61	0.08	0.50	0.66	0.02
NDF	51.95	45.65	51.86	45.36	48.10	2.56	0.08	0.94	0.48	0.05

¹Dietary treatments were low starch diet (LS0, without autolyzed yeast (*Saccharomyces cerevisiae*) product), high starch diet (HS0, without yeast), 15 g (HS15), 30 g (HS30), and 45 g of yeast (HS45) in a top dress, Top dress vehicle was 300 g of ground corn. LS0 and HS0 treatments received 300g of corn in a top dress.

²Contrasts were LS0 compared with HS0; and the linear, quadratic, and cubic effects of treatments (HS0, HS15, HS30, and HS45).

³Milk true protein N (milk true protein ÷ 6.38)

⁴Estimated from creatinine concentrations in spot urine samples assuming a creatinine excretion of 29 mg/kg BW (Valadares et al., 1999).

⁵Based on excretion of urinary purine derivatives (Valadares et al., 1999).

Table 3.3 Least squares means and associated SEM of the soluble fraction, degradable fraction, undegradable fraction, fractional rate of digestion (k_d), and effective degradability (ED) for alfalfa hay (AH). Bags were placed in the rumen of cows in a low starch diet without autolyzed yeast (AY) product (LS0), high starch diet without AY product (HS0), HS with 15 g (HS15), 30 g (HS30), and 45 g (HS45) of AY

Variable	Treatment ¹					SEM	P-value Contrasts ²			
	LS0	HS0	HS15	HS30	HS45		LS0 vs. HS0	Linear TRT	Quad TRT	Cubic TRT
Alfalfa Hay										
OM										
Soluble	0.31	0.31	0.33	0.31	0.30	0.01	0.60	0.17	0.30	0.33
Degradable	0.41	0.43	0.45	0.41	0.39	0.02	0.56	0.13	0.37	0.56
Undegradable	0.28	0.26	0.23	0.28	0.31	0.02	0.37	0.02	0.13	0.26
k _d , h ⁻¹	0.093	0.078	0.066	0.094	0.093	0.013	0.43	0.19	0.64	0.24
ED ³	0.54	0.52	0.52	0.53	0.52	0.01	0.19	0.87	0.63	0.44
NDF										
Soluble	0.17	0.19	0.21	0.18	0.17	0.02	0.38	0.32	0.37	0.42
Degradable	0.42	0.45	0.46	0.42	0.39	0.04	0.56	0.17	0.51	0.82
Undegradable	0.41	0.37	0.34	0.39	0.44	0.03	0.29	0.05	0.25	0.53
k _d , h ⁻¹	0.072	0.051	0.046	0.061	0.065	0.011	0.16	0.21	0.66	0.52
ED ³	0.39	0.37	0.37	0.38	0.36	0.01	0.25	0.63	0.31	0.39
ADF										
Soluble	0.24	0.24	0.26	0.24	0.25	0.01	0.80	0.96	0.64	0.36
Degradable	0.44	0.43	0.35	0.41	0.34	0.05	0.84	0.31	0.90	0.21
Undegradable	0.32	0.33	0.40	0.35	0.42	0.05	0.78	0.30	0.99	0.29
k _d , h ⁻¹	0.045	0.034	0.055	0.068	0.055	0.010	0.41	0.08	0.08	0.69
ED ³	0.39	0.37	0.40	0.38	0.38	0.01	0.16	0.89	0.17	0.46
CP										
Soluble	0.34	0.33	0.33	0.36	0.34	0.02	0.67	0.33	0.63	0.36
Degradable	0.53	0.54	0.57	0.51	0.51	0.02	0.73	0.14	0.55	0.12
Undegradable	0.13	0.13	0.10	0.14	0.15	0.01	0.97	0.20	0.11	0.14
k _d , h ⁻¹	0.086	0.082	0.068	0.083	0.080	0.012	0.81	0.87	0.59	0.38
ED ³	0.63	0.62	0.60	0.63	0.62	0.02	0.64	0.70	0.66	0.14

¹Dietary treatments were low starch diet (LS0, without autolyzed yeast (*Saccharomyces cerevisiae*) product), high starch diet (HS0, without yeast), 15 g (HS15), 30 g (HS30), and 45 g of yeast (HS45) in a top dress. Top dress vehicle was 300 g of ground corn. LS0 and HS0 treatments received 300g of corn in a top dress.

²Contrasts were LS0 compared with HS0; and the linear, quadratic, and cubic effects of treatments (HS0, HS15, HS30, and HS45).

³ Effective degradability (ED) = soluble fraction + degradable fraction $\times [k_d/(k_d+k_p)]$. Fractional rate of passage from the rumen (k_p) assumed to be 0.06.

Time points (TP) 0, 4, 12, 24, and 48h for AH.

Table 3.4 Least squares means and associated SEM of the soluble fraction, degradable fraction, undegradable fraction, fractional rate of digestion (k_d), and effective degradability (ED) for corn silage (CS). Bags were placed in the rumen of cows in a low starch diet without autolyzed yeast (AY) product (LS0), high starch diet without AY product (HS0), HS with 15 g (HS15), 30 g (HS30), and 45 g (HS45) of AY

Variable	Treatment ¹					SEM	P-value Contrasts ²			
	LS0	HS0	HS15	HS30	HS45		LS0 vs. HS0	Linear TRT	Quad TRT	Cubic TRT
<i>Corn Silage</i>										
OM										
Soluble	0.52	0.53	0.53	0.53	0.53	0.005	0.10	0.77	0.56	0.51
Degradable	0.35	0.28	0.26	0.25	0.27	0.03	0.05	0.73	0.54	0.92
Undegradable	0.13	0.19	0.21	0.22	0.20	0.03	0.11	0.79	0.45	0.88
k _d , h ⁻¹	0.034	0.043	0.057	0.054	0.045	0.009	0.44	0.97	0.21	0.78
ED ³	0.63	0.62	0.63	0.63	0.63	0.008	0.66	0.68	0.68	0.82
NDF										
Soluble	0.13	0.14	0.14	0.12	0.14	0.02	0.54	0.73	0.47	0.54
Degradable	0.75	0.37	0.47	0.46	0.40	0.10	0.007	0.85	0.41	0.87
Undegradable	0.12	0.48	0.40	0.43	0.48	0.10	0.009	0.95	0.41	0.82
k _d , h ⁻¹	0.021	0.046	0.085	0.053	0.069	0.019	0.34	0.66	0.49	0.10
ED ³	0.28	0.26	0.29	0.27	0.29	0.02	0.52	0.70	0.82	0.27
ADF										
Soluble	0.13	0.11	0.15	0.15	0.16	0.02	0.63	0.19	0.65	0.51
Degradable	0.66	0.43	0.54	0.35	0.43	0.08	0.04	0.65	0.90	0.08
Undegradable	0.21	0.46	0.31	0.51	0.43	0.08	0.02	0.76	0.65	0.06
k _d , h ⁻¹	0.028	0.034	0.026	0.042	0.049	0.011	0.69	0.20	0.43	0.46
ED ³	0.28	0.25	0.28	0.26	0.30	0.03	0.37	0.26	0.82	0.24
Starch										
Soluble	0.83	0.82	0.82	0.83	0.83	0.01	0.47	0.66	0.97	0.99
Degradable	0.16	0.17	0.17	0.17	0.17	0.01	0.81	0.92	0.89	0.96
Undegradable	0.003	0.005	0.002	0.004	0.003	0.001	0.26	0.23	0.38	0.08
k _d , h ⁻¹	0.167	0.173	0.205	0.182	0.165	0.028	0.87	0.69	0.38	0.63
ED ³	0.95	0.95	0.95	0.95	0.95	0.005	0.72	0.72	0.48	0.71

¹Dietary treatments were low starch diet (LS0, without autolyzed yeast (*Saccharomyces cerevisiae*) product), high starch diet (HS0, without yeast), 15 g (HS15), 30 g (HS30), and 45 g of yeast (HS45) in a top dress. Top dress vehicle was 300 g of ground corn. LS0 and HS0 treatments received 300g of corn in a top dress.

²Contrasts were LS0 compared with HS0; and the linear, quadratic, and cubic effects of treatments (HS0, HS15, HS30, and HS45).

³ Effective degradability (ED) = soluble fraction + degradable fraction $\times [k_d/(k_d+k_p)]$. Fractional rate of passage from the rumen (k_p) assumed to be 0.06.

Time points (TP) 0, 4, 12, 24, and 48h for CS.

Table 3.5 Least squares means and associated SEM of the soluble fraction, degradable fraction, undegradable fraction, fractional rate of digestion (k_d), and effective degradability (ED) for soybean meal (SBM). Bags were placed in the rumen of cows in a low starch diet without autolyzed yeast (AY) product (LS0), high starch diet without AY product (HS0), HS with 15 g (HS15), 30 g (HS30), and 45 g (HS45) of AY

Variable	Treatment ¹						P-value Contrasts ²			
	LS0	HS0	HS15	HS30	HS45	SEM	LS0 vs. HS0	Linear TRT	Quad TRT	Cubic TRT
Soybean Meal										
OM										
Soluble	0.44	0.44	0.43	0.44	0.44	0.006	0.68	0.61	0.22	0.52
Degradable	0.51	0.51	0.52	0.53	0.48	0.02	0.77	0.28	0.09	0.37
Undegradable	0.05	0.04	0.05	0.03	0.08	0.02	0.65	0.19	0.18	0.23
k _d , h ⁻¹	0.181	0.120	0.128	0.126	0.183	0.023	0.07	0.07	0.29	0.53
ED ³	0.79	0.76	0.76	0.78	0.78	0.01	0.12	0.33	0.79	0.40
CP										
Soluble	0.32	0.32	0.32	0.32	0.31	0.01	0.78	0.47	0.94	0.77
Degradable	0.62	0.65	0.65	0.66	0.61	0.03	0.48	0.41	0.31	0.43
Undegradable	0.06	0.009	0.01	0.02	0.03	0.01	0.001	0.13	0.80	0.82
k _d , h ⁻¹	0.164	0.095	0.103	0.101	0.138	0.021	0.03	0.15	0.53	0.58
ED ³	0.75	0.70	0.70	0.71	0.71	0.02	0.08	0.70	0.99	0.67

¹Dietary treatments were low starch diet (LS0, without autolyzed yeast (*Saccharomyces cerevisiae*) product), high starch diet (HS0, without yeast), 15 g (HS15), 30 g (HS30), and 45 g of yeast (HS45) in a top dress. Top dress vehicle was 300 g of ground corn. LS0 and HS0 treatments received 300g of corn in a top dress.

²Contrasts were LS0 compared with HS0; and the linear, quadratic, and cubic effects of treatments (HS0, HS15, HS30, and HS45).

³ Effective degradability (ED) = soluble fraction + degradable fraction $\times [k_d/(k_d+k_p)]$. Fractional rate of passage from the rumen (k_p) assumed to be 0.06.

Time points (TP) 0, 4, 12, and 24 for SBM.

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CHAPTER IV

AUTOLYZED YEAST TENDS TO INCREASE THE PERICELLULAR INTEGRITY OF RUMEN PAPILLAE, AND MAY IMPROVE OVERALL IMMUNE FUNCTION WHEN FED TO DAIRY COWS

ABSTRACT

Feeding diets high in starch for an extended period of time is known to elicit negative effects on the rumen and overall animal health in dairy cows. The high acid production in the rumen from highly fermentable carbohydrates can cause damage to ruminal epithelium if serve enough. However, with yeast supplementation this effect may not be as severe, yeast may even enhance dairy cow's performance. Therefore, selecting genes specific to inflammation and metabolism were chosen because of the changes that can occur between diet types. Fifteen rumen-cannulated Holstein cows were assigned to 1 of 5 treatments in a replicated 5×5 Latin square design balanced to measure carryover effects. Treatments were: low starch (**LS**) diet without autolyzed yeast (**AY**; control), high starch diet without AY (**HS0**), high starch diet with either 15 g (**HS15**), 30 g (**HS30**), or 45 g (**HS45**) of AY supplementation. Periods (21 d) were divided into the adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). During the measurement phase, rumen papillae and liver tissue biopsies were conducted on d 21 and then later extracted for total RNA. One single degree-of-freedom contrast LS0 compared to HS0 was used, and linear, quadratic, and cubic treatment effects of HS0, HS15, HS30, and HS45 for statistical inferences. Data were log-2 backtransformed. There were no differences for LS0 compared to HS0 for liver and rumen papillae gene expression. Yeast supplementation increased expression of *NFKB1* and *TLR4* with increased AY dosage (greatest expression at 0.99 and 1.05, respectively). There were no differences for genes associated with acute phase protein or metabolism associated genes in hepatic tissue. Additionally, for rumen papillae there was a

quadratic treatment effect for *CD14*, expressed greatest at HS15 (1.03). There was a cubic treatment effect for *TLR2*, expressed greatest at HS15 (1.00). Tight junction gene *CLDN1* tended to linearly increase expression with increased AY dosage. Cows in HS45 (0.96) had the greatest *CLDN1* expression compared other HS treatments. There were no differences for genes associated with metabolism in rumen papillae. In conclusion, cows supplemented with AY had greater *CD14*, *TLR2* and *CLDN1* rumen papillae gene expression when compared to HS0 and *NFKB1* and *TLR4* were greater expressed in hepatic tissue with the addition of AY compared to HS0.

Keywords: liver, rumen papillae, immune function, gene expression

INTRODUCTION

Dairy cattle fed high starch (**HS**) diets often experience deleterious effects when fed at high concentrations (> 25% of starch DM) for an extended period. These deleterious effects often lead to low ruminal pH, which can cause laminitis, diarrhea, decreased DMI, decreased milk production, altered milk composition, and, in extreme cases, liver abscess (Duffield, et al., 2004; Gozho et al., 2005; Krause and Oetzel, 2006; Plaizier et al., 2008). Cattle consuming highly fermentable CHOs can alter rumen function by rapidly increasing acid production. Increase in acid production in the rumen decreases ruminal pH and, if low enough, can elicit sub-acute ruminal acidosis, or SARA (Penner et al., 2007). Low ruminal pH or SARA for an extended period can lead to damage of the rumen epithelia (Dias et al., 2018). The functions of the rumen epithelia tissue includes metabolism, nutrient absorption, pH regulation, in addition to immune and barrier functions.

Damage to the rumen tissue will hinder cow performance and increase inflammation, for example the onset of liver abscess due to the release of toxins escaping through the rumen wall. These toxins have been attributed to free bacterial lipopolysaccharides endotoxin (**LPS**)

translocation from the rumen into blood circulation, then filtered through the liver and detoxified (Plaizier et al., 2008). This process can promote inflammation, eliciting changes on blood metabolites and gene expression in immune responsive tissues, such as liver and rumen papillae. Furthermore, it is reported cattle consuming high amounts of concentrate experience greater expression of inflammatory genes, like *IL1B*, *TLR4*, *TNF*, *MYD88*, and *NFKB1* in hepatic tissue (Xu et al., 2017). More so, Liu et al. (2013) reported profound changes occur in the rumen epithelial tight junctions (**TJ**) when ruminant consume high grain. The changes may promote impact on cellular functions and regulation. Primary barrier function proteins (*CLDN1* and *TJP1*) in rumen epithelial tissue had greater expression when cattle were exposed to increased starch (McCann et al., 2016) Similarly, Steele et al. (2011) observed less expression of tight junction *DSG1*, suggesting compromised rumen epithelial integrity with feeding a 65% grain diet. Therefore, in this study we postulated that the addition of AY supplementation would elicit changes in the rumen that may aid in better rumen conditions thus, improving the metabolic gene response in rumen papillae and hepatic tissue, and having a positive effect on the immune system.

MATERIALS AND METHODS

Animal Care and Housing

All experimental procedures were approved by the University of Illinois (Urbana-Champaign) Institutional Animal Care and Use Committee (#17172). The experimental period was from January through April 2018. Cows were housed in tie stalls with sand bedding, fed ad libitum, and had unlimited water access. Diet (TMR) was formulated to meet or exceed NRC (2001) recommendations for cows at 70 DIM, 703 kg of BW, producing 41 kg of milk/d with a

target 3.8% milk fat and 3.2% milk protein, and a predicted DMI of 25 kg/d (Knollinger et al., 2019, *submitted*).

Experimental Design

A total of 15 multiparous rumen-cannulated Holstein cows at the start of the experiment [BW (mean $\pm$ SD) = 623 $\pm$ 73 kg; DIM = 77 $\pm$ 26] were assigned to 1 of 5 treatments in a replicated 5 $\times$ 5 Latin square design balanced to measure carryover effects. Therefore, treatments were arranged so that the carryover effects could be evaluated. Periods (21 d) were divided into an adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). On d 1 to 21 of each period cows received 1 of 5 dietary treatments: low starch (**LS**) diet without autolyzed yeast (**LS0**) or HS diet without AY (**HS0**), 15 g of AY (**HS15**), 30 g of AY (**HS30**), or 45 g of AY (**HS45**). The AY (*Saccharomyces cerevisiae*) product was spray-dried and produced by an internal process technology for standardized autolytic degradation of the yeast cell (Levabon Rumen E, BIOMIN Holding GmbH, Austria). All cows were fed their respective diets once daily at 1400 h throughout the trial. The daily AY allocation was mixed with 300 g of ground corn and top-dressed onto the TMR immediately after feeding. Cows in LS0 and HS0 were given a top dress consisting of 300 g of ground corn only. The total consumption of the top dress was verified daily.

Data Collection and Sampling Procedures

Diet ingredient composition and nutrient analysis are explained elsewhere, in addition to production parameters (Knollinger et al., 2019, *submitted*). Total mixed ration offered and refused from each cow was recorded daily to determine intake based on weekly DM analyses.

Liver biopsies were conducted on d 21 for each period from each cow using a similar technique to that described by Vailati Riboni et al. (2015). An 18-gauge by 10.2 cm bone

marrow probe (Monoject-8881247087, Medtronic, Fridley, MN), was used to obtain approximately 2 g (wet weight) of liver sample. Liver samples were snap-frozen and stored in liquid nitrogen until further analysis.

Rumen papillae biopsies were conducted on d 21 for each period from each cow using a similar technique to that described in Steele et al. (2015). The rumen was partially emptied as needed through the rumen cannula into insulated trashcans to keep contents warm. The papillae were collected from the ventral sac, this site it determined to have the highest capillary blood flow per unit (Steele et al., 2015). Nail clippers and surgical scissors were used to clip approximately 300 mg (wet weight) of rumen papillae. The tissue was rinsed to remove any additional rumen contents. Rumen samples were snap-frozen and stored in liquid nitrogen until further analysis.

Hepatic Gene Expression

Briefly, total RNA was extracted and used for cDNA synthesis. All liver samples had an RNA integrity value greater than 7. Real-time quantitative PCR performed was SYBR Green-based, using a 7-point standard curve. The extraction and quantitative PCR analysis were preformed using previously established protocols at the University of Illinois (Vailati Riboni et al., 2015). Fourteen genes were used for transcript profiling in the hepatic tissue: apolipoprotein A5 (*APOA5*), ceruloplasmin (*CP*), carnitine palmitoyltransferase 1A (*CPT1A*), ELOVL fatty acid elongase 6 (*ELOVL6*), 3-hydroxy-3-methylglutaryl-CoA synthase 2 (*HMGCS2*), haptoglobin (*HP*), Interleukin 1, beta (*IL1B*), Interleukin-10 (*IL10*), nuclear factor κ B (*NFKB1*), phosphoenolpyruvate carboxykinase 1 (*PCK1*), serum amyloid A 3 (*SAA3*), signal transducer and activator of transcription 3 (*STAT3*), toll like receptor 4 (*TLR4*), and tumor necrosis factor

alpha (*TNFA*). All primers were created for the current experiment (Integrated DNA Technologies, Coralville, IA).

Rumen Papillae Gene Expression

Briefly, total RNA was extracted and used for cDNA synthesis. All rumen papillae samples had an RNA integrity value greater than 5.7. Real-time quantitative PCR performed was SYBR Green-based, using a 7-point standard curve. The extraction and quantitative PCR analysis were performed using previously established protocols at the University of Illinois (Vailati Riboni et al., 2015). Fourteen genes were used for transcript profiling in the rumen papillae tissue: cluster of differentiation 14 (*CD14*), claudin-1 (*CLDN1*), claudin-4 (*CLDN4*), desmoglein 1 (*DSG1*), 3-hydroxy-3-methylglutaryl-CoA synthase 2 (*HMGCS2*), Interleukin 1, beta (*IL1B*), Interleukin-10 (*IL10*), occludin (*OCLN*), solute carrier family 14 member 1 (*SLC14A1*), solute carrier family 16 member 1 (*SLC16A1*), transforming growth factor beta 1 (*TGFB1*), tight junction protein 1 (*TJP1*), toll like receptor 2 (*TLR2*), and toll like receptor 4 (*TLR4*). All primers were created for the current experiment (Integrated DNA Technologies, Coralville, IA).

RNA Extraction and Quality Evaluation

Total RNA was extracted using QIAzol® Lysis Reagent (Qiagen, Hilden, Germany). A total of 40 mg of tissue was placed in 1 mL of QIAzol®, homogenized with a Bead Beater (BioSpec, Bartlesville, OK) using two 30-sec cycle of the homogenizer at full speed, and placed on ice after every homogenization for one minute. Homogenized samples were centrifuged for 10 min at 12,000 g and 4 °C to remove any remaining cell debris. Chloroform was then added to homogenized sample, centrifuged for 15 min at 12,000 g and 4 °C, and aqueous phase carefully removed. Precipitation of RNA was achieved with the addition of ethanol (Decon Labs, Inc., King of Prussia, PA), and the subsequent RNA pellet was washed and cleaned using RNeasy mini spin

columns (Qiagen, Hilden, Germany). Genomic DNA was removed from RNA during purification with RNase-Free DNase Set (Qiagen, Hilden, Germany). The RNA concentration was measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies), while RNA quality was assessed using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).

mRNA cDNA Synthesis and qPCR Performance

All primers were created for the current experiment (Integrated DNA Technologies, Coralville, IA). A portion of the RNA was diluted to 100 mg/L using DNase/RNase free water prior to reverse transcriptase. cDNA was synthesized using 100 ng RNA per reaction, 1 µg dT18 (Operon Biotechnologies, Huntsville, AL), 1 µL 10 mmol/L dNTP mix (Invitrogen Corp., CA), 1 µL random primers (Invitrogen Corp., Carlsbad, CA), and 10 µL DNase/RNase free water. The mixture was incubated at 65 °C for 5 min and kept on ice for 3 min. A total of 6 µL of master mix composed of 4.5 µL 5X First-Strand Buffer, 1 µL 0.1 M DTT, 0.25 µL (50 U) of SuperScript™ III RT (Invitrogen Corp., Carlsbad, CA), and 0.25 µL of RNase Inhibitor (10 U, Promega, WI) was added. The reaction was performed in an Eppendorf Mastercycler® Gradient using the following temperature program: 25 °C for 5 min, 50 °C for 60 min and 70 °C for 15 min. cDNA was then diluted 1:4 (v:v) with DNase/RNase free water.

Quantitative PCR (**qPCR**) was performed using 4 µL diluted cDNA combined with 6 µL of a mixture composed of 5 µL SYBR Green master mix (Applied Biosystems, Foster City, CA), 0.4 µL each of 10 µM forward and reverse primers, and 0.2 µL DNase/RNase free water in a MicroAmp™ Optical 384-Well Reaction Plate (Applied Biosystems, Foster City, CA). Each internal control gene was run in triplicate and all samples in duplicate and a 7 point relative standard curve plus the non-template control (NTC) were used (User Bulletin #2, Applied Biosystems, Foster City, CA). The reactions were performed in an ABI Prism 7900 HT SDS

instrument (Applied Biosystems, Foster City, CA) using the following conditions: 2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C (denaturation) and 1 min at 60 °C (annealing + extension). The presence of a single PCR product was verified by the dissociation protocol using incremental temperatures to 95 °C for 15 s plus 65 °C for 15 s. Data were calculated with the 7900 HT Sequence Detection Systems Software (version 2.2.1, Applied Biosystems, CA). The extraction and qPCR analysis were preformed using previously established protocols at Dr. Loor's Lab at the University of Illinois. The final data were normalized using the geometric mean of 3 internal control genes for hepatic tissue: *GAPDH*, *RPS9*, and *UXT* (Khan et al., 2015) and the 3 internal control genes for rumen papillae: *CMTM6*, *ERC1*, and *MRPL39* (Minuti et al., 2015).

Statistical Analyses

Data collected from d 15 to 21 of each period was analyzed using SAS (v. 9.4, SAS Institute Inc., Cary NC). For variables the MIXED procedure of SAS was used to model the fixed effects of treatment, square, and period:

$$Y_{ijk} = \mu + T_i + S_j + P_k + \varepsilon_{ijk}$$

where Y_{ijk} = the observations for dependent variables; μ = the overall mean; T_i = the fixed effect of the i^{th} treatment; S_j = effect of the j^{th} square; P_k = effect of the k^{th} period; and ε_{ijk} = the random residual error. Cow was the experimental unit and considered as a random effect. The experimental design was balanced to measure carryover effects. Carryover effect was tested and was not present for any variable of interest ($P > 0.29$). One single degree-of-freedom contrast was used LS0 compared with HS0 along with the linear, quadratic, and cubic treatment effects for HS0, HS15, HS30, and HS45. Values reported are least squares means and associated standard errors of the mean. Degrees of freedom method was Kenward-Rogers (Littell, 2002).

Residuals distribution was evaluated for normality and homoscedasticity. The mRNA abundance data were log₂-scale transformed to fit normal distribution of residuals. Tables contain the log₂ back-transformed means that resulted from the statistical analysis. Statistical significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Performance

Production data is described at Knollinger et al. (2019, *submitted*). Briefly, Cows in HS0 had increased DMI (24.9 kg/d), BW (689 kg), and milk yield (34.1 kg/d) compared to LS0 (19.9 kg/d, 665 kg, and 30.9 kg/d, respectively; $P < 0.001$, $P = 0.003$, $P = 0.02$, respectively). Cows in HS0 had lower ruminal pH (6.10), nadir pH (5.53), and fecal pH (6.71; CONT1) when compared to LS0 (6.38, 5.74, and 6.95, respectively; $P < 0.001$, $P < 0.001$, $P = 0.042$, respectively).

Hepatic Gene Expression

Accession numbers, gene symbols, and forward and reverse sequences for all primers used are in Table 1. Hepatic gene expression response results are in Table 2. We observed no differences for LS0 compared to HS0 or quadratic treatment effects for liver gene expression. The expression of *NFKB1* and *TLR4* positively increased linearly ($P = 0.04$ and $P = 0.001$, respectively) with AY.

Rumen Papillae Gene Expression

Accession numbers, gene symbols, and forward and reverse sequences for all primers used are in Table 1. Rumen papillae gene expression response results are in Table 2. We observed no differences for LS0 compared to HS0 for rumen papillae gene expression. We observed a quadratic treatment effect for *CD14* ($P = 0.04$). In addition to a cubic treatment effect for *TLR2* ($P = 0.04$). Tight junction gene *CLDN1* tended to linearly increase expression

with increased AY dosage ($P = 0.08$). We observed no differences for genes associated with metabolism in rumen papillae.

DISCUSSION

In this study we postulated that the addition of AY supplementation would elicit changes in the rumen that may aid in better rumen conditions thus, improving the metabolic gene response in rumen papillae and hepatic tissue, and having a positive effect on the immune system. In the present study, we observed greater expression of *TLR4* in hepatic tissue with increased AY dosage. Toll-like receptors, such as *TLR2* and *TLR4*, serve as the first line of defense. Toll-like receptors, in particular *TLR4*, plays a key role in the innate immune system, are activated by different pathogen-associated molecular patterns (**PAMPs**), and help in identifying invading pathogens by binding to LPS located on the outer membrane of gram-negative bacteria (Murphy et al., 2012). However, LPS on the outer membrane of bacteria, can become unattached when an infection is present and bind to a protein known as LPS-binding protein, located in the blood, and extracellular fluid of tissue (Murphy et al., 2012).

Additionally, *CD14*, and *TLR2* had greater expression in rumen papillae with AY compared to HS0, greatest at HS15. Located on the surface of neutrophils, macrophages, and dendritic cells LPS can be transferred and bind to the protein *CD14* (Murphy et al., 2012). Furthermore, *NFKB1* in hepatic tissue increased expression with increased AY dosage. Toll-like receptors can activate numerous signaling pathways that then activate transcription factors, one in particular being *NFKB* (Murphy et al., 2012). The primary function of *NFKB* is to prompt pro-inflammatory cytokines, such as *TNFA*, *IL6*, and *IL1B* (Murphy et al., 2012). However, we didn't observe changes in any of these cytokines.

The modulation of immune cells through the release of pro-inflammatory cytokines from macrophages, has been reported to be stimulated by *S. cerevisiae* yeast cell wall components (Broadway et al., 2015). Naturally, inflammation helps the body overcome adverse challenges and aids in adaptation, with the objective of restoring homeostasis (Bradford et al., 2015). Furthermore, the occurrence of some inflammation is beneficial for future detection and to fight against later infections (Knoblock et al., 2019). Therefore, not all inflammation is negative. In the current study, we observed no health incidents, or hindrance on performance, indicative that this process was more a sign of functional immune system rather than a response to any infection or trauma. The haptoglobin concentration for the current study located elsewhere (Knollinger et al., 2019, *submitted*) but briefly, concluded plasma haptoglobin concentration below 20 ± 0.03 $\mu\text{g/mL}$ are considered without disease according to Tourlomoussis et al. (2004), the mean haptoglobin concentration for the current study was $10.63 \mu\text{g/mL}$ for cows supplemented with AY. Additionally, AY contains high availability of prebiotic bioactive nutrients, and yeast cell wall, which have been shown to modulate the immune response in animals by priming the adaptive and innate immune response via immune cell activation (Li et al., 2005; Volman et al., 2008). Taken together these findings may indicate a more robust immune response, therefore, supporting an improvement in immune function (Bradford et al., 2015).

Tight junctions are composed of transmembrane proteins (claudins and tight junction protein 1; **TJP1**) and their function is to seal adjacent epithelial cells and limit the passage of molecules and ions through the space between cells (Liu et al., 2013). Claudin- 1 (**CLDN1**) is located in the stratum granulosum and is one of the primary proteins associated with barrier function in rumen epithelium (Graham and Simmons, 2005). Deviations in the protein expression and distribution of the epithelial TJ barrier can lead to reduced structural integrity (Heiskala et

al., 2001; Schulzke and Fromm. 2009; John et al., 2011). Reduced TJ integrity causes increased epithelial permeability and elicits a “leaky” epithelial barrier; as a result, causing an inflammation response due to LPS and bacteria translocation (Turner 2009). Liu et al. (2013) reported goats fed HS diets experienced ruminal epithelial damage at the cell level and protein expression and distribution differences. In the current study, the expression of *CLDN1* increased with increased AY dosage, which may suggest, greater pericellular integrity of the ruminal papillae barrier. Knoblock et al. (2019) reported no differences for mRNA tight junction expression between HS diet (27%) or LS diet (21.6 %) treatments and *S. cerevisiae* supplementation. Furthermore, with improvements in tight junction integrity structure with AY supplementation, this strengthens the argument that cows were not affected by inflammation but instead improved immunity.

Similar to the current study, Dias et al. (2018), reported no treatment differences for *SLC16A1* in a HS diet (29%), LS diet (23%), or with the addition of yeast supplementation. However, Dias et al. (2018) did report that cows in the HS treatment tended to have decreased expression of *SLC14A1* and *TLR4* when compared to cows in the LS diet. Genes *SLC14A1* and *SLC16A1* are associated with mediating the transepithelial transport of VFA, ketones, and lactate from the rumen (Dias et al., 2018). Similar to Dias et al. (2018), perhaps the dietary treatment differences in the current study were not enough to provoke metabolic changes in VFA influx on rumen epithelial.

CONCLUSION

In conclusion, in the present study, greater expression of *TLR4* and *NFKB1* in hepatic tissue with AY supplementation, and greater expression of *CD14*, and *TLR2* in rumen papillae with the addition of AY, suggests activation of an immune response. In rumen papillae, TJ

CLDN1 improved with AY supplementation. Together, indicating AY may increase the pericellular integrity of the rumen papillae barrier, and may improve overall immune function when fed to dairy cows. Although more research is needed to assess the originators of this process, a variety of signals may contribute from a HS diet, AY supplementation, blood biomarkers, and the changes in rumen environment. Further investigation of measuring LPS in the blood may aid in further discussion of mechanisms occurring and aid in greater explanation of gene expression for rumen papillae and hepatic tissue.

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TABLES

Table 4.1 Accession number, gene symbol, and forward and reverse primer sequences (5' 3' used in real time PCR) of genes analyzed in liver tissue and rumen papillae

Accession no.	Symbol	Forward sequence	Reverse sequence
Liver genes			
NM_001083492.2	<i>APOA5</i>	CTGCGATCTGAGCCTCTAACAA	CCTTTGGTTGGCCTCAGCTAT
NM_001256556.1	<i>CP</i>	GGTTGACACGGAACATTCCAA	GGCCTAAAAACCCTAACCAGACA
FJ415874	<i>CPT1A</i>	CAAAACCATGTTGTACAGCTTCCA	GCTTCCTTCATCAGAGGCTTCA
BC148954.1	<i>ELOVL6</i>	AGCACCCGAAGTAGGAGATACAAT	TACCAGGAGTACAGAAGCACAGTGA
NM_001034034.2	<i>GAPDH</i>	TGGAAAGGCCATCACCATCT	CCCACCTTGATGTTGGCAG
NM_001045883.1	<i>HMGCS2</i>	TTACGGGCCCTGGACAAAT	GCACATCATCGAGAGTGAAAGG
NM_001040470.2	<i>HP</i>	GGTTCGGAAAACCATCGCTA	CACTCGTGTCCTCCCTCCCTC
NM_174093.1	<i>IL1B</i>	ATTCTCTCCAGCCAACCTTCATT	TTCTCGTCACTGTAGTAAGCCATCA
XM_027564331.1	<i>IL-10</i>	GAAGGACCAACTGCACAGCTT	AAAACCTGGATCATTTCCGACAAG
NM_001076409.1	<i>NFKB1</i>	TTCAACCGGAGATGCCACTAC	ACACACGTAACGGAAACGAAATC
JQ733520.1	<i>PCK1</i>	AAGATTGGCATCGAGCTGACA	GTGGAGGCACTTGACGAACTC
NM_001101152.2	<i>RPS9</i>	CCTCGACCAAGAGCTGAAG	CCTCCAGACCTCACGTTTGTTT
NM_181016.3	<i>SAA3</i>	GGGCATCATTTTCTGCTTCCT	TTGGTAAGCTCTCCACATGTCTTTAG
NM_001012671.2	<i>STAT3</i>	GGTAGCATGTGGGATGGTCTCT	GCATCCCTAGAACTCTGGTCAA
NM_174198.6	<i>TLR4</i>	TGCGTACAGGTTGTTCTAACATT	TAGTTAAAGCTCAGGTCCAGCATCT
NM_173966.3	<i>TNFA</i>	CCAGAGGGAAGAGCAGTCCC	TCGGCTACAACGTGGGCTAC
BC108205.1	<i>UXT</i>	TGTGGCCCTTGATATGGTT	GGTTGTCGCTGAGCTCTGTG
Rumen papillae genes			
NM_174008.1	<i>CD14</i>	CACCACATTGCACACCTGTT	CACCACATTGCACACCTGTT
NM_001001854	<i>CLDN1</i>	GGCATCCTGCTGGGACTAATAG	CAGCCATCCGCATCTTCTGT
NM_001014391	<i>CLDN4</i>	CCCCAGCCAGCAACTACGT	TCACAGATTGCAGTGAGCTCAGT
NM_001035066.1	<i>CMTM6</i>	TTCACCTTTGACACATGACAATACCA	CACGGAGCATAAAGGAGAACTCA

Table 4.1 (cont.)

Accession no.	Symbol	Forward sequence	Reverse sequence
NM_001013004	<i>DSG1</i>	AGACAGAGAGCAATATGGCCAGT	TTCACACTCTGCTGACATACCATCT
NM_001205419.1	<i>ERC1</i>	CCTCCCATTCCGGTCAAAG	GTCTGATGTACAACTTGAGCTTGCTT
NM_001045883.1	<i>HMGCS2</i>	TTACGGGCCCTGGACAAAT	GCACATCATCGAGAGTGAAAGG
NM_174093.1	<i>IL1B</i>	ATTCTCTCCAGCCAACCTTCATT	TTCTCGTCACTGTAGTAAGCCATCA
XM_027564331.1	<i>IL-10</i>	GAAGGACCAACTGCACAGCTT	AAAACCTGGATCATTTCGACAAG
NM_017446.3	<i>MRPL39</i>	AGGTTCTCTTTTGTGGCATCC	TTGGTCAGAGCCCCAGAAGT
NM_001082433.2	<i>OCN</i>	GCCATTTTCGCCTGTGTTG	CCAAAGGCACTTCCTGCATAA
NM_001008666.1	<i>SLC14A1</i>	GATAAAGAAGCCTCCGGTTGTG	AAGCTTCCCACTGCTGTATTCAG
NM_001109980.1	<i>SLC16A1</i>	CCTGTGGGACTGAAGGGTAAAT	ATGATTCCCACAGAAATGTCCAGTAT
NM_001166068.1	<i>TGFB1</i>	CGTGGAGCTGTACCAGAAATATAGC	CAACTCCAGTGACGTCAAAGGA
XM_002696650.3	<i>TJP1</i>	GCACATAGGATCCCTGAACCA	TGCTTCCGGTAGTACTCCTCATC
NM_174197.2	<i>TLR2</i>	CCATGTCTGGAGAGGGTGTT	GGGGACACAAAACAGCACTT
NM_174198.6	<i>TLR4</i>	TGCGTACAGGTTGTTCTAACATT	TAGTTAAAGCTCAGGTCCAGCATCT

Table 4.2 Least squares means and associated SEM for hepatic and rumen papillae mRNA abundance in Holstein cows (n = 3/treatment), log-2 backtransformed. Treatments are low starch diet without autolyzed yeast product (LS0), high starch diet without autolyzed yeast product (HS0), HS with 15 g (HS15), 30 g (HS30), and 45 g (HS45) of autolyzed yeast during the experimental period. Samples were collected on d 21 during the last week of each period

Variable	Treatment ¹					SEM	P-value Contrasts ²			
	LS0	HS0	HS15	HS30	HS45		LS0 vs. HS0	Linear TRT	Quad TRT	Cubic TRT
Liver genes ³										
Inflammation										
IL1B	0.94	0.97	0.97	1.02	0.98	0.03	0.35	0.48	0.36	0.19
IL-10	0.99	0.90	0.91	0.97	0.91	0.06	0.70	0.56	0.38	0.31
NFKB1	0.97	0.96	0.97	0.98	0.99	0.01	0.59	0.04	0.98	0.84
STAT3	0.97	0.97	0.98	0.95	1.02	0.03	0.81	0.88	0.56	0.14
TLR4	1.01	0.99	1.00	1.04	1.05	0.02	0.28	0.001	0.96	0.41
TNF	0.97	0.99	0.98	1.04	1.00	0.05	0.71	0.43	0.66	0.22
Acute-phase proteins										
CP	0.99	0.94	0.97	0.96	0.97	0.03	0.11	0.36	0.70	0.68
HP	0.50	0.43	0.59	0.42	0.36	0.24	0.55	0.21	0.17	0.24
SAA3	0.88	0.82	0.91	0.85	0.85	0.06	0.16	0.80	0.14	0.18
Metabolism										
APOA5	0.99	1.00	0.97	1.03	1.02	0.02	0.49	0.19	0.40	0.06
CPT1A	0.92	0.92	0.89	0.92	0.91	0.2	0.90	0.86	0.54	0.18
ELOVL6	0.98	1.01	0.98	1.01	1.04	0.04	0.45	0.30	0.21	0.62
HMGCS2	1.02	1.01	1.01	1.04	1.03	0.02	0.81	0.21	0.89	0.35
PCK1	1.01	1.02	0.99	1.00	0.97	0.03	0.75	0.16	0.89	0.44
Rumen papillae genes ⁴										
Inflammation										
CD14	0.98	0.97	1.03	1.01	1.01	0.02	0.75	0.24	0.04	0.11
IL1B	0.94	0.85	0.88	0.85	0.93	0.08	0.21	0.33	0.58	0.21
IL10	0.95	1.00	0.92	0.96	0.98	0.08	0.39	0.95	0.29	0.50
TLR2	0.97	0.97	1.00	0.96	0.99	0.02	0.98	0.65	0.86	0.04
TLR4	0.96	0.96	0.99	0.98	0.97	0.02	0.96	0.55	0.16	0.49
Tight junction										
CLDN1	0.93	0.93	0.95	0.95	0.96	0.02	0.77	0.08	0.55	0.56
CLDN4	0.94	0.97	1.02	0.96	0.97	0.03	0.32	0.56	0.40	0.12
DSG1	0.84	0.98	0.99	0.85	0.95	0.08	0.90	0.14	0.69	0.26
OCLN	0.96	0.98	0.97	0.98	0.99	0.02	0.21	0.73	0.53	0.84
TJP1	0.94	0.95	0.94	0.94	0.96	0.01	0.56	0.28	0.11	0.82
Metabolism										
HMGCS2	0.98	1.01	0.98	1.01	1.01	0.03	0.19	0.79	0.42	0.21
SLC14A1	0.84	0.88	0.85	0.88	0.87	0.03	0.24	0.84	0.57	0.30
SLC16A1	0.88	0.90	0.88	0.89	0.88	0.03	0.41	0.62	0.80	0.60
TGFB1	0.93	0.92	0.92	0.90	0.94	0.02	0.80	0.75	0.18	0.23

¹Dietary treatments were low starch diet (LS0, without autolyzed yeast (*Saccharomyces cerevisiae*) product), high starch diet (HS0, without yeast), 15 g (HS15), 30 g (HS30), and 45 g of yeast (HS45) in a top dress. Top dress vehicle was 300 g of ground corn. LS0 and HS0 treatments also received 300 g of ground corn in a top dress.

²Contrasts were LS0 compared with HS0; and the linear, quadratic, and cubic effects of treatments (HS0, HS15, HS30, and HS45).

Table 4.2 (cont.)

³Liver samples that were analyzed were collected on d 21 of each period. ILB = Interleukin 1, beta; IL10 = Interleukin-10; NFKB1 = nuclear factor kappa B1; STAT3= signal transducer and activator of transcription 3; TLR4= toll like receptor 4; TNF = tumor necrosis factor alpha; CP = ceruloplasmin; HP = haptoglobin; SAA3 = serum amyloid A 3; APOA5 = apolipoprotein A5; CPT1A = carnitine palmitoyltransferase 1A; ELOVL6 = ELOVL fatty acid elongase 6; HMGCS2 = 3-hydroxy-3-methylglutaryl-CoA synthase 2; PCK1 = phosphoenolpyruvate carboxykinase 1.

⁴Rumen Papillae samples that were analyzed were collected on d 21 of each period. CD14 = cluster of differentiation 14; CLDN1 = claudin-1; CLDN4 = claudin-4; DSG1 = desmoglein 1; HMGCS2 = 3-hydroxy-3-methylglutaryl-CoA synthase 2; ILB = interleukin 1, beta; IL-10 = interleukin-10; OCLN = occludin; SLC14A = solute carrier family 14 member 1; SLC16A1 = solute carrier family 16 member 1; TGFB1 = transforming growth factor beta 1; TJP1 = tight junction protein 1; TLR2 = toll like receptor 2; TLR4 = toll like receptor 4.

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CHAPTER V

OVERALL SUMMARY, CONCLUSIONS, AND PERSPECTIVES

Reduction in milk prices can bring economic pressures on dairy farmers. With a loss in profits, the average dollar goes a little shorter these days. Especially with feed costs being the most expensive expenditure on the farm. Improvements such as maximizing animal efficiency through increasing digestibility will aid in decreased energy disbursement and more nutrient availability. Furthermore, increased environmental regulations on pollution have created new pressures for nutrient use improvements for the agricultural industry. It is important to ensure the profit from the cow is greater than the expense. Thus, it is pertinent to increase herd efficiency and improve animal productively while still minimizing environmental pollution.

From this study, we gained better knowledge about AY supplementation when used with HS diets that will better help dairy producers maximize animal potential and productivity. We observed that when cows are fed a high starch diet, AY supplementation improved DMI and milk production efficiencies. The addition of AY had positive impacts on rumen pH, as well as plasma concentrations of superoxide dismutase and total protein. When cows are fed a high starch diet, AY supplementation improved fecal N excretion and CP digestibility, in addition to having positive impacts on the rate of degradation, and increased starch and OM in situ digestibility. At the gene expression forefront, we observed greater expression of *CLDN1* therefore, less permeability of ruminal papillae. Greater expression of *TLR4* and *NFKB1* in hepatic tissue with AY supplementation indicates an inflammation process was occurring. Similarity, *CD14* in rumen papillae increased with the addition of AY. Thus, together these findings suggest an improvement in immune function. Overall, the addition of autolyzed yeast helped alleviate the negative implications of high starch diets fed to Holstein cows.

Further research, investigating fatty acids in milk may explain the greater milk fat concentration for cows in the LS0 treatment when compared to HS0 and measuring LPS in the blood, may elucidate metabolic processes that better help explain gene expression differences in rumen papillae and hepatic tissue.

APPENDIX A. MATERIAL AND METHODS FOR DNA EXTRACTION AND RUMEN MICROBIOME

Rumen Sampling Collection

Rumen fluid and content samples were collected via rumen cannula on d 15 and 16 in relation to feeding at 1400 h (time point 0). Samples were extracted at 8 h. Rumen samples were collected and composited from 3 sites (ventral sac, cranial sac, and caudo-ventral blind sac). Whole rumen samples were mixed by hand and squeezed through 2 layers of cheesecloth before immediately analyzing rumen fluid for pH (AP115, Fisher 103 Scientific, Pittsburgh, PA). A 50 mL subsample of squeezed rumen contents and 15 mL of rumen fluid were snap-frozen and stored at -80°C .

DNA Extraction for Microbiome Analysis

Briefly, rumen fluid and rumen content 8 h time point was used for total DNA extraction similar to Yu and Morrison 2004 using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Valencia, CA, USA), and bead-beating method for lysis of bacterial cell walls. The processing of rumen content solids before extraction was based on Stevenson and Weimer (2007) with some modifications. A 10 g of solid rumen content was used for extraction. To each sample was added 40 mL of chilled Extraction Buffer (EB), which was composed of 100 mM Tris/HCl, 10mM ethylenediaminetetraacetic acid (EDTA), and 0.15 M NaCl at pH of 8.0. The mixture was blended by polytron for 2 min. The probe of the polytron blender was then rinsed continuously until free of ruminal contents and debris with tap water and then Mili-Q water. The resulting mixture was then vortexed and filtered through 4 layers of cheesecloth into a clean 50 mL Conical tube and centrifuged gently at $500 \times g$ for 5 min at 4°C to remove the considerable amount of broken plant particles while keeping bacterial cells in suspension. The resulting supernatant was centrifuged at $16,000 \times g$ for 10 min at 4°C and the supernatant was

discarded. The resulting pellet from the solid and the liquid samples, which contained bacterial cells, were re-suspended in 2 mL ice-cold EB buffer. A volume of 2500 μ L was aliquoted into two 1.5 mL microfuge tubes. The re-suspended pellet was frozen at -20°C for later usage. A total of 300 μ L of the re-suspended pellet from the rumen contents and 250 μ L of rumen fluid were used in the DNA extraction protocol. Subsequently, to each sample 25 g of zirconia beads (0.1 and 0.5 mm diameter) and 1.0 mL of lysis buffer. The samples were homogenized, shaken in a bead beater for 3 min, incubated at 70 °C for 15 min, and then centrifuged at 16,000 *g* for 15 min at 4°C. The supernatant was transferred to a fresh 2 mL microcentrifuge tube, and the above step was repeated, pooling the supernatant together.

Next, precipitation of nucleic acids was performed by adding 260 μ L of 10 mM ammonium acetate to each microcentrifuge tube and then vortexed. Samples were incubated on ice for 5 min and centrifuged at 16,000 *g* for 10 min at 4°C. The supernatant was transferred to two 1.5 ml microcentrifuge tubes and 1.0 volume of isopropanol was added and vortexed. Samples were then incubated at room temperature for 30 min then centrifuged at 16,000 *g* for 15 min at 4°C, and supernatant was decanted. Adding 1 ml of 70% ethanol and inverting the tube end-over several times. Centrifuging the samples at 16,000 *g* for 5 min at 4°C, then decanting the supernatant. Samples were spun in mini centrifuge and the remaining liquid was pipetted out. The nucleic acid pellets were dissolved in 100 μ L of warm TE buffer and vortexed, pooling the two nucleic acid solutions from the same digesta sample.

Lastly, the following steps were performed to remove RNA, protein and DNA purification. Therefore, 2.0 μ L of DNase-free RNase was added, vortexed gently and then incubated at 37°C for 15 min. Furthermore, 15 μ L of proteinase K and 200 μ L of buffer AL was added, mixed and incubated at 70°C for 10 min. The addition of 200 μ L of ethanol was added

and mixed. All the liquid was transferred to QIAamp columns and centrifuged at 16,000 *g* for 1 min, the flow-through was discarded. Additionally, 500 µl of buffer AW1 was added and centrifuged for 1 min at room temperature, flow-through was discarded, and 500 µl of buffer AW2 was added and centrifuged for 1 min. The columns were placed in a fresh 2 ml collection tube and centrifuged at room temperature for 1 min to dry. The columns were then placed in a pre-labeled 1.5 ml collection tube and 75 µl of warm TE buffer was added into each column. Finally, the samples were incubated at room temperature for 2 min, centrifuged (12,000 *g*) at room temperature for 1 min to elute the DNA, and repeat the elution into the same tube resulting in 150 µl. The extracted DNA was stored at -20 °C until diluted with DNase/RNase free water to 20 ng/µL of DNA and analyzed. A microbiome test kit was performed using ZymoBIOMICS Microbial Community Standard (Zymo Research, CA). The DNA concentration was measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies), while DNA quality was assessed using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). A total of 150 samples were sequenced for 16s rRNA amplicon. The V3 through V4 region of the 16S rRNA gene was amplified with F357/R805 primers using the Fluidigm Access Array (Fluidigm Corp, South San Francisco, CA) and sequenced on the HiSeq platform (2 × 250 bp; Illumina, San Diego, CA) at the University of Illinois, W. M. Keck Center for Biotechnology (Urbana, IL). Additionally, four primers were chosen Archaea, 18s, fungal, and protozoa.

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