Spring 2020

Preservation and Utilization of Wet Brewer's Grains in Diets of Dairy Heifers

Eric Hatungimana
University of New Hampshire, Durham

PRESERVATION AND UTILIZATION OF WET BREWER'S GRAINS IN DIETS OF DAIRY HEIFERS

By

ERIC HATUNGIMANA

B.S., National University of Rwanda, 2006

M.S., University of Arkansas, 2010

A DISSERTATION

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences

May 2020

This dissertation has been examined and approved in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Agricultural Sciences by:

Dr. Peter S. Erickson, Dissertation Director
Professor of Dairy Nutrition and Management
University of New Hampshire, Durham

Dr. Andre F. Brito
Professor of Dairy Cattle Nutrition and Management
University of New Hampshire, Durham

Dr. Drew Conroy
Professor of Dairy Management
University of New Hampshire, Durham

Dr. Susan Marston
Professor of Animal Sciences
University of Massachusetts, Amherst

Dr. Gbenga Ayangbile
Vice President of Research and Laboratory Services
Agri-King, Fulton, Il

Original approval signatures are on file with the University of New Hampshire Graduate School.

ACKNOWLEDGEMENTS

I thank the Almighty God for all the blessings He has given me. I am who I am today because of His unlimited Grace and Mercy.

I would like to thank the Department of Agriculture, Nutrition and Food Systems and the University of New Hampshire for offering me this great opportunity to pursue my education and provide all financial support needed to gain both teaching and research experience that are relevant to my future career.

My heartfelt thanks go to my major advisor Peter Erickson. You went beyond the call of duty to provide me with mentoring, training and many opportunities to better make myself as a scientist while I was doing my Ph.D. program under your guidance. Your passion for the dairy industry, your expertise, and hard-working ways will always inspire me. You helped me to grow both personally and professionally. You have been always on my side during this long and hard journey. Your kindness, sincere support, and understanding will always be remembered.

I thank the USDA National Institute of Food and Agricultural Project (Hatch Multistate NC-2042) to have financially supported my research experiments.

I highly thank my committee members Gbenga, Susan, and Andre for serving on my committee and for your guidance and support. Your constructive criticism, suggestions, and explanations have been instrumental to the completion of my doctoral program.

Special thanks go to Drew, the Instructor of CREAM for which I have been the Teaching Assistant for 4 years. CREAM has been such a great experience of learning and applying dairy science to the real world. You helped to get integrated into the new environment which is different

from where I grew up. You took me to many places, and I met many people who became friends of mine, and I did not feel lonely during my 4-year stay in the US.

To Patrick, Mohamad, Kayla, Meaghan, Tess, and Luiz, I would not have overcome the difficulties I encountered in this journey without your help as my fellow graduate students.

I thank the Fairchild Dairy farm crew for all the help provided when I was conducting my research experiments.

To my lovely wife Judith, my son Angelo and my daughter Erica. You have been so patient while I was away to pursue my studies. Whenever I was coming home after a year, it has been always a great reunion and joy to meet the family I truly love. You encouraged me by face-talking to me every single day and I did not feel alone while being thousands of miles away.

To my parents, sisters, and brothers as well as close friends, thank you for your encouragements.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

PRESERVATION AND UTILIZATION OF WET BREWER'S GRAINS IN DIETS OF DAIRY HEIFERS

By

Eric Hatungimana

University of New Hampshire, May 2020

Three experiments were conducted. The objective of Experiment 1 was 2-fold: 1) to evaluate the effect of storage of wet brewer's grains (WBG) treated with salt or a commercial preservative (PRES) on yeast and mold growth and in vitro dry matter digestibility (IVDMD) and 2) to evaluate in situ dry matter (DM) and protein digestibility of WBG treated with salt and PRES. Seven treatments were used for objective 1: control (0%), PRES (0.05%, 0.10%, 0.15%); or salt (1.4%, 2.6%, 3.8%) and were allocated randomly in duplicate to 14 plastic tubs containing of 48.5 kg of fresh WBG. For objective 2, 3 cannulated cows were used twice (same 3 cows) in a replicated 3×3 Latin square. The WBG was treated with 0% or 0.10% PRES or 2.6% salt and preserved for 1 wk before being used for the in situ experiment. Results showed that WBG treated with PRES had the least yeast counts. The WBG treated with salt (3.8%) and PRES (0.15%) had the least mold counts. Greater IVDMD was observed in WBG treated with salt. Treatment with 2.6% salt resulted in greater in situ DM and protein digestibility. Experiment 2 evaluated the growth performance and apparent total-tract nutrient digestibility of limit-fed diets containing WBG to Holstein heifers. Treatments were 0%, 10% and 20% of WBG on a DM basis, and diets were formulated to be limit-fed for dry matter intake (DMI) at 2.35% of body weight (BW) and provided 15% crude protein (CP) and 2.27 Mcal ME / kg of DM. Results demonstrated that DMI, BW,

average daily gain (ADG) were not different among treatments. Skeletal measurements and body condition scores (BCS) were not different among treatments except for the change in heart girth and initial BCS. Apparent total- tract digestibilities of DM, organic matter (OM), CP, fat and hemicellulose were greater or tended to be greater in heifers fed 0% and 20% WBG treatments than heifers fed 10 % WBG. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and fat digestibilities were similar among treatments. During the digestibility phase, DMI tended to be greater for the 10% WBG treatment. Experiment 3 evaluated the effect of storage of WBG with incremental levels of salt on total-tract nutrient digestibility and purine derivative excretion in dairy heifers. Treatments were 0%, 0.8%, 1.6% and 2.4% salt added to fresh WBG and stored for 4 days before being included in the diet at 20% on the DM basis. Diets (DM basis) were formulated to be limit-fed at 2.15% of BW to provide 14% CP and 2.27 Mcal ME/kg of DM. Results demonstrated that DMI was greater in heifers fed 1.6% salt diet while BW, ADG and feed efficiency were greater in heifers fed diets containing WBG treated with salt. Digestibility of DM, OM and CP linearly decreased with increasing levels of salt in WBG. Urinary volume, allantoin, and uric acid excretion and total purine derivative excretion were not affected by treating WBG with salt. Mold and yeast counts were not different among treatments. The losses in WBG weight (fresh basis) linearly decreased as salt increased.

CHAPTER I

REVIEW OF LITERATURE

Part I: Wet Brewer's Grains

Feed is the largest single production expense for dairy cattle operations. With the current decrease in milk prices and increased feed cost especially corn and soybean-based concentrates, it has been imperative for dairy producers to look for alternative feed sources that are cheaper and nutritious to meet nutritional requirements and improve animal performance. Moreover, there is currently a great political and social pressure to mitigate pollution arising from industrial activities. Consequently, most industries are looking for ways to turn residues into reusable raw material for other processes (Mussatto et al., 2006).

In many cases, agro-industrial by-products such as wet brewer's grains (**WBG**) have been reported to be a valuable feed source for dairy cattle (Rounds and Klopfenstein, 1975; Herrington et al., 1983; Polan et al., 1985). Wet brewer's grains are high in protein, ruminal undegradable protein (RUP), fiber, and energy content. It is a cheaper alternative compared to higher-cost feed grains (Westendorf et al., 2014). According to Merchen et al. (1979), WBG possess two potential advantages as a protein source for ruminants: firstly, it constitutes a source of slowly degradable protein, which bypasses the rumen to a large extent and secondly its combination with urea provides rumen microbes with sufficient nitrogen (N) for their optimum growth while minimizing N losses absorbed as ammonia from the rumen.

Generation of Wet Brewer's Grains

The brewing industry generates large amounts of by-products and wastes; WBG spent hops and yeast being the most common (Mussatto et al., 2006). Wet brewer's grains are the most abundant brewing by-product corresponding to about 85% of the total by-products (Reinold, 1997). On average, WBG accounts for 31% of the original malt weight representing approximately 20 kg per 100 L of beer produced (Reinold, 1997).

The method of brewing beer is an important factor in the WBG nutrient composition. Despite the similarity of the brewing process across most breweries, an overview of beer production is necessary to understand subsequent WBG production. Mussato et al. (2006) describes it in the following steps: in preparation for brewing, harvested barley grains are cleaned and graded according to size. After a dormancy period of 4-6 weeks, barley grains are malted in a controlled germination process which serves to increase the enzymatic content of the grain. The malting process is performed through steeping, germination and drying or kilning. During steeping, barley grains are soaked in warm water at between 5 and 18° C for approximately 2 days when the grain moisture content reaches between 42 and 48%.

As Mussato et al. (2006) continues to describe, the steeping water must be changed every 6-8 h. Hydration during steeping initiates the germination and activates aleurone metabolism. After steeping, barley grains are conveyed to germination vessels where they are regularly turned and maintained in contact with a humid air stream flowing through the grain bed at a temperature between 15 and 21° C. The main purpose of germination is to promote the synthesis and activation of enzymes in the aleurone and starchy endosperm such as amylases, proteases, β-glucanases, etc. (Woonton et al., 2005). The germination process of barley grains takes 6 or 7 days after which

they are dried or kilned at 40-60° C with a moisture content of 4 to 5% to avoid any microbial contamination and to produce flavor components.

After approximately 3 to 4 weeks of storage, the malted barley grains are milled, mixed with water in the mashing vessel with increasing temperature from 37 to 78° C to promote enzymatic hydrolysis of starch, proteins, β-glucans, arabinoxylans and to solubilize their breakdown products (Kendal, 1994). During the mashing process, starch is converted to fermentable sugars mainly maltose and maltotriose and non-fermentable sugars (dextrins) while proteins are partially degraded to polypeptides and amino acids (Lynch et al., 2016). At the end of the mashing process, a sweet liquid known as wort is produced. The undegraded portion of the malted barley grains is allowed to settle while the wort is filtered and used as the fermentation medium to produce beer (Linko et al., 1998).

The residual solid fraction is known as WBG mainly made of water-insoluble proteins, cell walls of the husk, pericarp and seed coat but also can include adjuncts (non-malt sources of fermentable sugars) such as wheat, rice or corn added during mashing (Reinold, 1997). It is this by-product that can be utilized by dairy producers as a feed source.

Chemical composition and physicochemical properties of WBG

The chemical composition of WBG depends on the barley variety, harvest time, malting, mashing conditions and quality and type of adjuncts added in the brewing process (Huige, 1994). Wet brewer's grains are considered as a lignocellulosic material rich in protein and fiber, which accounts for around 20 and 70% of its composition respectively (Mussato et al., 2006).

According to Mussato et al. (2006), WBG consists of the husk-pericarp-seed coat layers that covered the original barley grain. The starch content of WBG is negligible because most of it

is removed during the mashing process. Residues of hops introduced during mashing may be present in WBG depending on the brewing technique.

In summary, the major constituents of WBG are cell walls of the husk-pericarp-seed coat, which are rich in cellulose and non-cellulose polysaccharides and lignin and may contain some protein and lipid. The husks of WBG contain a large amount of silica and much of the polyphenolic components of the barley grain. Around 25% of the mineral content of the barley is present as silicates. The mineral concentrations are lower than 0.5% and those include calcium, cobalt, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium and sulphur (Huige, 1994). The vitamin composition (mg/kg) of WBG include biotin (0.1), choline (1800), folic acid (0.2), niacin (44), pantothenic acid (8.5), riboflavin (1.5), thiamine (0.7); protein-bound amino acids leucine, valine, alanine, serine, glycine, glutamic and aspartic acid in the largest amounts, and tyrosine, proline, threonine, arginine, and lysine in small amounts (Huige, 1994).

Protein and fiber content of WBG are high because most of the starch is removed during mashing (Kissel and Prentice, 1979). The NRC (2001) estimated the CP value of WBG at 28.1% for WBG which makes it a useful supplemental protein source (Murdock., 1981). However, the CP value or other nutrients can vary somewhat between breweries depending on the beer processing technology used.

As a high protein source, WBG can potentially be used to substitute concentrate, more specifically soybean meal (SBM) or corn. NRC (2001) breaks protein into nitrogen fractions, A, B, and C depending on the rate of degradability. Fraction A is made of protein that is readily degradable in the rumen to be primarily incorporated into microbial protein as amino acids, peptides, nitrates, nitrites, and non-protein nitrogen (NPN) that includes nucleic acids, ammonia, and urea. The B fraction is more slowly degraded than fraction A or can pass to the small intestine

as rumen undegradable protein (RUP) depending on the rate of passage. Fraction C is not digested in the rumen or the small intestine and will pass the entire digestive system without being used at all. Comparing the protein fractions for SBM (A: 22.5%, B: 76.8%, C: 0.7%) to that of WBG (A: 48.3%, B: 42.5%, C: 9.2%), there is a clear difference among those fractions (NRC, 2001). Soybean meal contains more slowly degradable protein fraction (B fraction) than that of WBG, making it a great source of protein for ruminal microbes but also a source for RUP depending on the rate of passage (Armentano et al., 1986). For WBG, about half of protein is degraded in the rumen to provide amino acids for microbial protein synthesis.

Two related fiber components of great importance for WBG are neutral detergent fiber (NDF) and acid detergent fiber (ADF). The neutral detergent fiber is defined as the sum of hemicellulose, cellulose, and lignin, whereas ADF is made of cellulose and lignin. The difference between NDF and ADF being the hemicellulose content. According to NRC (2001), WBG contains approximately 47.1% NDF and 23.1% ADF. The fiber content of any feedstuff is important for ruminal health, milk and milk fat composition, as well as DMI. Effective fiber is crucial to stimulate salivary buffer production to maintain a proper ruminal pH and helps to prevent ruminal metabolic disorders such as acidosis. The amount of dietary fiber can affect DMI as well as digestibility depending on what type of fiber source is being used (West et al., 1994). Wet brewer's grains, despite being considered as a supplemental protein source, they are also considered as a fiber source for ruminants (Hersom, 2006).

Another nutrient component of WBG is ether extract (EE) commonly known as crude fat. According to the NRC (2001), both wet and dried brewer's grains have an EE value of about 5.2% DM. Fat provides more energy than carbohydrates but feeding diets containing above 5 - 6% fat which are unsaturated may lead to milk fat depression and reduced feed digestibility (Dhiman, et

al., 1999). The gross and net energy of WBG was reported to be 20.14 MJ/kg and 18.64 MJ/kg (Russ et al., 2005).

Spoilage of WBG and different methods of preservation

The preservation of feeds aims to inhibit the growth of undesirable microorganisms that would cause spoilage of feedstuffs while minimizing losses of nutrients and energy (Aragon, 2012). The main limiting factor for effective utilization of WBG is its high moisture content and fermentable sugars which make it an unstable feed source and may spoil quickly, leaving most breweries only with local market opportunities (Johnson et al., 1987). The storage of WBG under aerobic conditions, as commonly used in most farms, provides ideal conditions for the development of microorganisms such as filamentous fungi (molds) and yeasts, promoting the degradation of the by-product stored under these conditions (Allen et al., 1975).

Yeasts are eukaryotic unicellular aerobic micro-organisms that use OM as a source of energy, mainly from hexoses and disaccharides, and do not need sunlight to grow (Aragon, 2012). Yeasts grow well in a neutral or lower acidic environment and can grow very fast and cause aerobic instability during the feed-out phase if no preservatives such as acetic or propionic acids are used. Feeds that contain yeasts are harmful to animals as they have the potential to cause diarrhea (Alugongo et al., 2017). During fermentation, yeasts compete with lactic acid bacteria for sugars and produce ethanol which has very little preservation effect and causes DM and energy losses (Rotz and Muck ,1994).

Molds whose majority are strictly aerobic, grow in multicellular filaments and get their energy from OM. Molds compete with other micro-organisms by secreting mycotoxins and hydrolytic enzymes. Ingestion of feeds contaminated with mycotoxins have been reported not only

to decrease animal performance in terms of milk production and reproduction but also to be the source of other diseases such as mastitis, laminitis and gastro-intestinal disorders (Aragon, 2012).

The inoculation of WBG by spoilage microorganisms such yeast and mold occurs from different sources that include processing equipment, spoiled feed around the storage pad, environment hygiene, and transportation equipment (Kung, 2005). The growth rate for yeasts and molds on wet grain is dictated by different factors. The temperature has a great influence on the growth of yeasts as most of them grow well between 25° C to 40° C, and their growth slows down in cool weather (Kung, 2005). Another factor affecting the growth rate of spoilage microorganisms is water available for metabolic activity (a_w). Wet brewer's grains have greater a_w that causes rapid growth of microbes. Moreover, residual sugar and starch in high-moisture by-products such as WBG or wet distiller's grains provide enough nutrients to stimulate spoilage microorganisms. Several techniques have been proposed to increase the shelf-life of WBG:

Preservation of WBG with organic acids

Organic acids play a role as both acidifiers and antimicrobial agents that can change silage fermentation. Their acidity is lower than that of inorganic acids, but they are very effective due to their antimicrobial property to control fermentation (Kung et al., 2003). The main organic acids that have been studied include formic acid, propionic, lactic, acetic, acrylic, benzoic, and sorbic acids (Woolford, 1984).

Al-Hadithi et al. (1985); evaluated lactic, formic, acetic or benzoic and formic acid-water-WBG mixtures packed in plastic containers held for 3 summer months and reported that all effectively preserved WBG quality and nutritional value with the most effective being benzoic and formic acids. Potassium sorbate was effective in preserving pressed WBG (Kuntzel and

Sonnenberg, 1997). Propionic acid is more effective at inhibiting the growth of molds than that of yeasts but when propionic acid is ammoniated, it is relatively active to inhibit the yeast growth (Kung, 2005). However, undissociated acids are more effective due to their ability to pass through the cell membranes and liberate their protons, thus acidifying the cytoplasm and decreasing the proton gradient.

Organic acids have been combined in an effort of having synergetic effects on the aerobic stability of silages. Razavi-Rohani and Griffiths (1999) found that ethylenediamine-tetra-acetic acid (EDTA) improved the antimycotic effect of sorbic and propionic acid. It was also reported that adding a mixture of 0.5 g/kg each of sorbate and EDTA was effective as 1.0 g/kg of benzoic acid to enhance the aerobic stability of corn silage (Kleinschmit, 2002). Queiroz et al. (2018, cited Woolford, 1975), reported that sorbate and benzoate are effective preservative that mitigate the growth of Bacillus spp. However, the undissociated form of sorbate has the antimicrobial effect and its pKa is higher than that of benzoate, which makes sorbate to be more effective (Kung et al., 2003). Other chemical additives such as sodium benzoate, sodium nitrite, hexamine and sodium propionate can be used to inhibit clostridia in silages (Queiroz et al. (2018, cited Jonsson et al. 1990).

Drying

Drying WBG provides an advantage of reducing the product volume and therefore, decreases the transport and storage costs (Santos et al., 2003). The traditional technique for drying WBG uses the rotary-drum driers, a technique that requires high energy cost but has an advantage to reduce protein degradation in the rumen and increase amount of protein that flows to the duodenum, provided that microbial protein synthesis is not impaired (Armentano et al., 1986;

Pereira et al., 1998). The high cost associated with drying has resulted in increased feeding of WBG (Johnson et al., 1987)

Bartolomé et al. (2002) evaluated three drying methods for preserving WBG: freeze-drying, oven drying, and freezing. Freezing technique is not effective because it requires the storage of large volumes of WBG and its content of arabinose may be altered.

Oven drying reduces the volume of WBG, but it must be conducted at temperatures < 60° C to avoid unpleasant flavors that are generated by high temperatures (Prentice and D'Appolonia, 1977; Hernandez et al., 1999). Moreover, during oven drying, the grain temperature near the dryer exit may lead to toasting or burning, or the smoke emerging from dryer stacks may cause air pollution (Huige, 1994). Heat treatment of WBG above optimal temperature may result in over protection of protein to a level where it is neither degraded in the rumen nor digested in the small intestine (Dakowski et al., 1996)

Using superheated steam would be an alternative drying technique to save energy, reduce environmental impact, improve drying efficiency and eliminate explosion risk or enhance the recovery of important organic compounds (Tang et al., 2004). Freeze drying decreases the volume of the by-product and does not alter its composition. However, freeze-drying is economically not feasible (Baltolome et al., 2002).

Another method is to use a membrane filter press in which WBG is mixed with water and filtered at a feed pressure of 3 to 5 bar (1 bar = 100 kilopascals), washed with hot water (65°C), membrane-filtered, and vacuum-dried to reach moisture content of about 20 to 30% (El- Shafey et al., 2004).

Ensiling or storing with dried feeds

Ensiling is an ancient technique of preserving the nutritive value of feed by packing and storing feed in airtight conditions in which lactic acid bacteria (**LAB**) convert sugars into mainly lactic acid, but also acetic or butyric acid is produced under anaerobic conditions (Rooke, 2004). The combination of WBG with drier feed decreases its moisture content and helps to reduce a potentially large amount of seepage (Kung, 2005). When high-moisture by-products are ensiled with dry feeds such as straws, beet pulp, bran and hulls as a TMR, the risk of effluent production was reduced and the time for mixing before feeding was eliminated. Moreover, unpalatable by-products could be incorporated into TMR to mask their odor and flavors if altered by silage fermentation (Nishino et al., 2003). Among many absorbents used to reduce the loss of effluents from WBG, straw was shown to be more effective but the digestibility of straw/WBG was the lowest (Harrison, 1996).

Ensiling with bacterial inoculants

Silage additives are used to preserve the nutritive value of feed in case circumstances could impair proper fermentation. Silage microbial inoculants stimulate fermentation and help to complement the native lactic acid bacteria to maintain a proper fermentation (Muck, 2008), but also increasing DM and nutrient recovery and extending aerobic stability (Queiroz et al. (2018). According to McDonald et al. (1991), microbial inoculants have been used for many decades to improve silage fermentation and stability. Wilkinson (1999), cited by Queiroz et al. (2018), reported the most common pathogenic microorganisms found in silage to be *Escherichia coli*, particularly *E. coli* O157:H7, *Listeria monocytogenes*, *Bacillus* spp., *Salmonella*, and *Clostridium* spp.

Microbial inoculants are divided into 2 groups according to how they ferment sugar content available in feed: Homofermentative inoculants are often based on *Lactobacillus plantarum, Lactobacillus casei, Pediococcus* spp, and *Enterococcus* and have the main role to produce lactic acid and increase the rate of pH reduction in the early fermentative phase of ensiling. Homofermentative inoculants also help to preserve protein from degradation, reduce ammonia formation and improve silage palatability (Muck, 1993). Homofermentantive LAB (lactic acid bacteria) are efficient to eliminate *Bacillus* spp. and other harmful bacteria and enhance homolactic lactic fermentation (Queiroz et al., 2018).

Heterofermentative inoculants are based on *Lactobacillus buchneri* and produce lactic acid, acetic acid, ethanol, and carbon dioxide. Heterofermentative inoculants have been introduced recently and seemed to be more efficient in laboratory-scale studies (Kleinschmit and Kung, 2006). While homofermentative inoculants enhance fermentation quality, heterofermentative inoculants enhance aerobic stability (Kung et al., 2003).

Compared to corn silage, by-products such as WBG may be difficult to ensile alone due to high moisture content and lack of sugar substrates, the main cause of increase in butyric and acetic acid production especially with a prolonged storage as well as a quick spoilage when by-products are exposed to air after the silo is opened (Imai, 2001).

In a study by Allen and Stevenson (1975), it was reported that bacterial inoculants may improve the ensilability of WBG as they observed a rapid increase in the lactobacilli population which caused a faster pH decline, increased concentration of lactate and reduced concentrations of acetate and butyrate. According to Lowes et al. (2000), a successful fermentation is indicated by a rapid decline in pH as lactic acid bacteria convert soluble sugars into lactic acid and a small amount of acetic acid or no butyric acid.

The combination of both types of homofermentative and heterofermentative inoculants would be more advantageous in sense of having a fast initial pH reduction controlled by homofermentative bacteria while good aerobic stability if controlled by heterofermentative bacteria that produce more acetic acid (Fransisco et al., 2009)

Marston et al. (2009), compared the deterioration of WBG when Silo-King GPX (a commercial preservative containing different strains of bacterial inoculants), was added to either covered or non-covered piles of WBG and found that adding Silo-King at a rate of 0.45/900kg of WBG at time of delivery may maintain nutritive value up to 2 weeks and reduce subsequent spoilage.

In regard with ensiling ensiling WBG with bacterial inoculants, it is imperative to prevent contamination before and after ensiling the feed material by setting adequate control measures and ensure management practices that favor rapid homolactic fermentation so that the rapid drop in pH inhibits the growth of clostridium spp. and enterobacteria. Queiroz et al. (2018), reported that LAB such as *L. plantarum, Pediococcus acidilacti, P. pentosaceous*, and *Enterococcus faecium* are efficient to inhibit harmful bacteria and increase the rate of acidification in silages.

Potential applications for Wet Brewer's Grains

Wet brewer's grains in ruminant nutrition

Brewer's grains are commonly marketed or fed in two forms, wet (WBG) and dry (DBG). Wet brewer's grains are obtained directly from the brewery without drying and DBG continue the processing including the separation of the brewers condensed solubles and drying (Westendorf et al., 2002). According to NRC (2001), WBG and DBG have similar chemical composition, both have a similar crude protein content of 28-29% but can have differences within each N fraction.

Brewer's grains have been successfully included in diets fed to lactating dairy cows (Davis et al., 1983; Armentano et al., 1986). In a study by Belibasakis and Tsirgogianni (1996), it was reported that feeding WBG at 16% of the total ration DM (substituted for corn silage, **SBM** and wheat bran for lactating cows in hot weather significantly increased milk yield, 4% fat-corrected milk yield, milk fat content and milk total solids. Such positive responses on milk yield and fat content result from WBG which is a good source of the limiting amino acids methionine and lysine, and **RUP** made of high-quality protein for milk production (Clark et al., 1987). Similarly, Hoffman and Armentano (1988) evaluated the substitution of 23.1% WBG for SBM in early lactating cows and found the same DMI in both groups and a trend of higher milk yields with cows fed WBG compared to SBM.

Polan et al. (1985), fed diets supplemented with either WBG or DBG to multiparous Holsteins and cows supplemented with DBG and WBG produced more milk than cows supplemented with SBM and cows fed the basal diet. The level at which WBG is added to diets could have adverse effects when DMI is decreased. Davis et al. (1983); observed decreased DMI when cows were fed 30 and 40% of WBG on DM basis (replacing SBM in the concentrate mixtures), but overall performance was similar for cows fed 0 and 20% WBG. However, West et al. (1994) found no difference in milk yield when cows were fed WBG up to 30% of the DM replacing ground corn and SBM in the diet.

Wet brewer's grains have been also fed to beef cattle and it was reported that including lower levels of WBG could support the growth performance and carcass characteristics of finishing cattle (Parmenter et al., 2018). Moreover, Shand et al. (1998), recommended feeding WBG or wet distiller's grains as alternative protein sources to provide lean, nutritious beef for consumer' s satisfaction.

Wet brewer's grains in non-ruminant nutrition

According to Prentice et al. (1978), feeding WBG to livestock may provide some health benefits because of their dietary fiber content which affects non-infectious diseases. Thus, incorporating WBG into swine and poultry diets may help reduce constipation and diarrhea, due to the high content in glutamine-rich protein, and non-cellulosic polysaccharides and small amounts of β-glucans (Tang et al., 2009). Wet brewer's grains can also be incorporated into fish diets and provide similar growth performances as feeds containing corn, soybean meal, groundnut cake, cottonseed meal, dried brewery yeast, and palm kernel meal (Nwokolo, 1986). Kaur and Saxena (2004), reported that incorporation of WBG at 4 levels (10, 20, 30 and 40%) in supplementary fish feed, replacing rice bran at 25, 50, 75 and 100 % respectively increased body weight gain for fish fed 30% brewery waste.

Moreover, as WBG is derived from material used by humans, it has been successfully incorporated into several bakery products such as bread, cookies, muffins, snacks, mixed grain cereals, fruits and vegetables, cakes, waffles, pancakes, tortillas, doughnuts, and brownies, (Huiges, 1994). It was added to these products to boost their fiber contents resulting in the prevention of some diseases such as cancer, gastrointestinal disorders, diabetes and coronary heart diseases (Stojceska et al., 2008). However, due to its granular form, WBG must first be converted to flour before direct addition in food (Hassona, 1993). Consumption of WBG-derived products provides other health benefits that are associated with increased fecal weight, accelerated transit time, increased cholesterol and fat excretion and a decrease in the prevalence of gallstones (Fastnaught, 2001). Such health effects are attributed to the content of glutamine-rich protein, and the high content of cellulosic polysaccharides (arabinoxylan and small amounts of 1 - 3, 1 - 4 β-glucans; Vietor et al., 1993).

Wet brewer's grains in metal adsorption and immobilization

Plant wastes and agro-industrial by-products have been used and considered as the cheapest and unconventional adsorbents for heavy metals from aqueous solutions (Li et al., 2009). In a study by Lu and Gibb (2008), WBG was used to remove Cu (II) from aqueous solution with the maximum adsorption capacity of 10.47 mg/ g dry weight at pH 4.2. Based on those results, WBG has significant potential as a bio adsorbent to remove metal-contaminated wastewater streams. Moreover, when functional groups such as hydroxyl, amine, and carboxyl are activated in WBG, they can potentially bind metal ions (Li et al., 2009). The adsorption of heavy metal using WBG can be enhanced if WBG is pretreated with 0.5 M NaOH solution and Low et al. (2000) reported higher adsorption capacity of 17.3 and 35.5 mg/g for cadmium and lead respectively compared to the control.

Wet brewer's grains in bioethanol production

Bioethanol is produced from crops with high starch (sweet sorghum, maize, wheat, etc.) and sugar-based crops (sugar cane) as well as lignocellulosic biomass. However, there is a high competition of those crops with human food production and their high production prices restrict their industrial production (Aliyu and Bala, 2011).

Today, there is a need to search for abundant substrate and development of an efficient and less expensive way to produce ethanol (Alam et al., 2009). The composition of WBG in grain husks, hemicelluloses, cellulose, and lignin, makes it a potential feedstock to make ethanol through current advances to convert residues using chemical or enzymatic hydrolysis to produce fermentable sugars followed by microbial fermentation (Aliyu and Bala, 2011). Xiros et al. (2008)

reported ethanol yield of 74 and 109 g/kg of dry WBG by microbial fermentation using *Neurospora crassa* and *Fusarium oxysporum* under microaerobic conditions respectively.

Wet brewer's grains as a growth medium for microorganisms and enzyme production

The high moisture, polysaccharides and protein content of WBG attract microbial growth and degradation. Wet brewer's grains have been used to cultivate different strains of microorganisms such as *Lactobacillus sp*., *Streptomyces, Penicillium sp., Bifidobacterium adolescentis,* etc. (Novik et al., 2007; Szponar et al., 2003; Panagiotou et al., 2006). Hence WBG is a suitable medium for isolation, maintenance of different microbial strains to produce new biologically active substances and fast spore production. Digestible and non-digestible organic residues of WBG make it a potential substrate on which amylolytic organisms could be cultured to produce β-amylase, amyloglucosidases, xylanases, feruloyl esterase and α-L-arabinofuranosidases (Adeniran et al., 2008).

Part II: Methods of measuring digestibility for the nutritional evaluation of feeds

Many factors determine the nutritive value of feeds such as composition, odor, texture, and taste (Schneider and Flat, 1975). The digestibility of feeds simply determines the amount of feed absorbed by the animal and not recovered in feces, therefore the availability of nutrients for growth, production, reproduction, etc., (Cochran and Galyean, 1994). The combination of digestibility and intake data is very useful to make an accurate prediction of overall nutritive value. Of the two factors, intake is more important than digestibility in predicting overall nutritive value because highly digestible feeds are of less value unless ingested by the animal (Khan et al., 2003). However, digestibility provides a reliable index of feed nutritive value because greater feed

consumption is highly correlated with more digestible than less digestible feeds (Khan et al., 2003). Several techniques for measuring digestibility have been investigated:

Total collection technique

This method also known as conventional digestion trial is the most reliable to measure feed digestibility, but it is expensive, time-consuming and laborious. With this technique, the animal is restrained in an individual metabolic chamber or tie stall, fed a feed in known quantity, and a total collection of feces is made (Khan et al., 2003). This implies accurate records of feed intake, refusals, and fecal output and a sub-sample of each is collected for analysis. When N balance is of interest, urine output is also measured. The animals in the experiment are allowed from 7 to 21 d of adaptation to the feed followed by a collection period. Collected samples are then dried, ground and analyzed for desired nutrients. Nutrient digestibility can be calculated as follows:

$$\text{Nutrient digestibility (\%)} = \frac{\text{Nutrient intake} - \text{Nutrient in feces}}{\text{Nutrient intake}} \times 100$$

The use of metabolic chamber for the total collection of feces has been criticized for a low or abnormal feed intake resulting from animal discomfort (Khan et al., 2003). Therefore, the total collection of feces in tie stall is used by placing portable wooden boxes at the end of each stall covering the rear area accessible by each cow in the experiment, so feces can only be evacuated in the boxes (Ghelichkhan et al., 2018).

Marker technique

The marker technique has been developed to reduce the time and expense involved in digestion experiments using methods in which total feces are not collected and weighed but only analyzed. Kotb and Luckey (1972), designated the new method as the indicator or index method.

In this technique, apart from the chemical analysis of nutrients, an indigestible reference substance is also analyzed. The substance is considered as an internal marker when is a natural constituent of the feed (lignin, silica, acid insoluble ash, indigestible ADF) or is considered as an external marker when it is added to the feed (ferric oxide, chromic oxide, etc.) (Van Keulen and Young, 1977; Waller et al., 1980).

A good marker must not be absorbed, must not affect or be affected by the gastrointestinal tract or its microbial population, must be perfectly associated with the feed material and must be sensitive and specific to its method of estimation and not interfere with other analyses (Khan et al., 2003). The digestibility is then calculated from the relation between the nutrients and the indicator substance in the feed and the feces. The DM can be computed with the following equation:

$$\text{DM digestibility (\%)} = 100 - 100 \times \frac{\%\ \text{Indicator in feed DM}}{\%\ \text{Indicator in fecal DM}}$$

When the percentage of any nutrient in the feed and in the feces is known, as well as the indicator in the feed and in feces, the digestibility of that nutrient can be easily calculated with the following equation:

$$\text{Nutrient digestibility (\%)} = 100 - 100 \times \frac{\%\ \text{Indicator in feed} \times \%\ \text{Nutrient in feces}}{\%\ \text{Indicator in feces} \times \%\ \text{Nutrient in feed}}$$

With this technique, it is assumed that the used marker will pass throughout the gastrointestinal tract at a uniform rate and only a small amount of feces is enough to provide a good estimate of digestibility.

Prediction technique

The prediction of digestibility from chemical analysis of a feed involves the development of multiple regression equations that relate different chemical components to in vivo digestibility. However, digestibility estimates from the prediction equations are not accurate (± 3 to 4%) compared with values obtained from conventional trials (total collection), (Khan et al., 2003).

In vitro technique

The in vitro digestibility method is quite simple but subject to many variables that may influence the digestibility results (Khan et al., 2003). In vitro digestibility is measured by substrate disappearance when feeds are incubated with ruminal fluid. The commonly used method is a two-stage method adapted from the technique originally described by Tilley and Terry (1963). To proceed, a small sample of ground feed (0.5 g) is weighed into a 50 ml centrifuge tube, a buffer and a ruminal fluid from a donor animal are added together and the tube is incubated for 48 h at 39°C. The fermentation is stopped, and tubes are centrifuged, and supernatant fluid discarded. The residue is then digested in acidified pepsin for another 48 h at 39° C to match digestion in the abomasum. The final content is filtered, and the residue is dried and weighed.

The drawback of this technique is that it uses cannulated animals to get ruminal fluid and has long incubation periods. Even if the technique is based on the premise that the final residue is comparable to the feces voided by animals eating the feed, this assumption is not strictly correct because metabolic N, which is present in vivo but not in in vitro residues, can decrease protein digestibility estimates in vivo (Khan et al., 2003). The residues from the in vitro technique may contain bacterial residues which would have been digested in the distal parts of the digestive tract.

Nylon bag technique to measure digestion kinetics

The nylon bag technique consists of using nylon bags (pore size of 2µm) of approximately 5 cm x 15 cm, which are filled with 2 to 3 g of ground feed and then incubated in the rumen of a cannulated cow. The bags must be secured with a weighted cord to prevent floating in the rumen and allow exposure to microbial digestion (Khan et al., 2003). After the incubation period, bags are removed, washed under tap water, dried and weighed to determine DM remaining. For this technique, adequate pore size (50 µ or less) is desired to prevent the passage of feed out of the bag (one of the disadvantages). Moreover, the sample to bag size ratio of approximately 10 mg / cm^2 is also important. Through multiple incubation times, the nylon bag is important to evaluate kinetic aspects of digestion in ruminants.

Gas technique

The gas production technique simulates in vivo fermentation of feedstuffs and accounts for contributions from soluble and insoluble feed fractions. Moreover, this technique provides measures of microbial growth when nutrient content is not limiting (Khan et al., 2003). However, this technique has some drawbacks such as the dependence of total gas production on sample size, sample form and the composition of end products of fermentation (Tilley and Terry, 1963).

Feeds differing in chemical composition produce different proportions of volatile fatty acids (**VFA**) and the ratio of fermented to degraded carbohydrate and yield of gaseous products per mole of hexose fermented are not constant (Khan et al., 2003). The interpretation of gas production is complicated by the reaction of end products of fermentation with the gas produced, as such indirect gas production is accounted for. Appropriate models should be used to estimate the parameters of the ruminal fermentation curve (Merchen, 1988).

In vitro systems to investigate rumen fermentation

Batch culture

Batch cultures are used to estimate digestibility or extent of degradation in the rumen using either a single end-point or kinetic parameter measurement or fermentation end-product accumulation (Weiss, 1994). Batch cultures are mostly used for experiments that have a large or small number of samples or experimental treatments must be screened before the trial (Tamminga and Williams, 1998). The problem of using batch cultures in rumen fermentation studies is that only short to medium experiments (hours or days) are feasible and the microbial growth pattern does not allow to reach the steady-state conditions. The shortening of fermentation substrate and the accumulation of waste products result in the decrease or death of microbial population (Lopez).

Continuous culture

According to Czerkawski (1986), the continuous culture is more efficient as it allows a regular addition of buffer and nutrients and a continual removal of end-products of fermentation, and this allows the stability of microbial population for a long period. In this system, measurements of fermentation parameters, extent of DM degradation, output of end products of fermentation and microbial synthesis are feasible. The continuous culture is divided into three types according to Czerkawski (1991): the first is the semi-permeable type in which a continuous dialysis system uses a microbial culture enclosed in a semi-permeable membrane. Due to its complexity, this system is not suitable for routine use. The second is the continuous culture type in which a liquid buffer solution containing nutrients is continuously infused, and feed substrate is regularly dispersed into the vessel, and some of the mixture can overflow. The dual flow system considers of a dual effluent removal system in which the differential flow of liquids and solids is simulated (Hoover et al.,

1976). The third is the Rusitec (Rumen simulation technique) in which there is a single outflow to control dilution. In this system, there is a continuous infusion of the buffer solution and removal of the liquid effluent but there is no continuous feed supply (Czerkawski and Breckenridge, 1977). The advantage of Rusitec is that it simulates the compartmentation occurring in the rumen and the kinetic studies are easy to be conducted (Czerkawski, 1986). According to Hannah et al. (1986), Rusitec and dual flow are excellent in vitro methods for studying ruminal microbial fermentation as they almost simulate rumen conditions.

Factors affecting feed digestibility

The digestibility of feed can be influenced by the following factors:

Feed intake

Many experiments have shown that livestock fed a restricted amount of feed digest a larger proportion of nutrients than when they are fully fed (Okin and Mathison, 1991; Faichney, 1993). The depression of apparent digestibility with a large intake is caused by a rapid flow rate of feed through the gastrointestinal tract, thus allowing less time for digestion and absorption of nutrients.

Particle size

When a feed is ground to a very fine particle size, digestibility is decreased due to a high rate of passage that consequently decreases the digestibility (Firkins et al., 1986; Galloway et al., 1993).

Chemical composition

The chemical composition is the most significant factor that affects feed digestibility (Sarwar et al., 1985; Luginbuhl et al., 1994). The chemical composition of a feed affects how the

enzymes interact with the substrates to digest. Generally, forage high in crude fiber has a poor digestibility. The addition of small amounts of protein or soluble carbohydrates can enhance the digestibility of feeds (Khan et al., 2003).

Feed processing

Changing the physical form of a feed may affect the digestibility of its organic substance such as protein, carbohydrate or its DM in general (Sarwar et al., 1992). Feed processing includes drying, grinding, pelleting or chopping all affect digestibility. Feed processing can also include chemical or biological treatment to enhance fibrous feed digestibility (Sarwar et al., 1994).

Environmental temperatures

Feed digestibility has been observed to be greater with high temperatures than in cold environments due to the mean retention of feedstuffs in the digestive tract (Faichney, 1986). In a study conducted by Kennedy (1985), sheep exposed to freezing temperature (0°C) exhibited lower digestibility than controls in warm temperatures (22°C), and this was probably due to increased reticulo-rumen motility that caused a decreased mean retention time of feed in the digestive tract. Such a greater rate of passage could serve as a strategy to increase DM consumption to meet greater energy requirements imposed by cold weather (Merchen, 1988).

Animal age

In the case of ruminants, young animals cannot digest much fiber or roughage to a greater extent until their rumen is fully developed (Khan, 2003). However, the ability of older animals to digest feeds may be decreased with poor teeth making chewing activity difficult. Besides, other factors may affect digestibility such as frequency of feeding, amount of water consumed, animal

species, etc., but data are contradictory, and more research is needed on these factors (Khan et al., 2003).

Part III: Raising replacement dairy heifers

The heifer of today will be the cow of tomorrow. This statement by Le Cozler et al. (2008) implies that a successful dairy heifer feeding, and management is not only measured in terms of daily gains or BW at calving but also must be measured in terms of lifetime milk production capacity. It is important to understand the impact of nutritional management for the development of a heifer to be efficient and profitable.

The goal of a successful dairy heifer program is to have an optimal growth to calve between 22 and 24 months of age while minimizing inputs (feed, time, labor) and nutrient excretion (Akins, 2016), without compromising future production potential, health, or welfare (Hoffman et al., 1997). Moreover, it is important to mention that raising dairy heifers is an expensive operation (second or third largest contributor) because of income absence until the onset of lactation (Zanton and Heinrichs, 2009). Hence, feeding practices are required to enhance the economic stability, environmental sustainability, and physiological efficiency of dairy heifers.

Heifer development

According to Davis and Drackley (1998), raising a healthy calf begins during gestation with proper management and nutrition of the dam for the entire gestation period. It is during the last trimester (from d 190) of gestation that the developing fetus requires greater nutrients, and this implicates a proper feeding of the prefresh cow to provide not only enough nutrients to maintain a positive energy balance and prevent metabolic disorders in the dam but also providing enough nutrients for the growing fetus (Bell, 1995). During gestation, the calf in utero does not get

maternal immunoglobulins (Ig) because of the 6-layer epitheliochorial placenta, and this dictates the ingestion of colostrum is so important for the new calf for her immunity against diseases.

Smith and Little (1922) first discovered the importance of colostrum in the survival and health of the newborn calf when calves were either fed colostrum or deprived of colostrum. During the first 3 weeks of life, mortality rates were 0% for calves fed colostrum while 75% in calves deprived of colostrum. Calves that were deprived of colostrum died of septicemia which indicates that colostrum supplied important components to prevent infections in newborn calves. A few years later, Ratner et al. (1927) suggested that calves lacked protective agglutinins (later called Ig) in plasma at birth due to the placental structure. Stott et al. (1979) determined that the Ig in the calf plasma was increased after consumption of colostrum.

Many studies were then conducted to elucidate more importance of colostrum in the newborn and indicated that calves that do not consume enough amounts of colostrum have reduced growth rates, increased risks of infections leading to high mortality and morbidity, increased risks of being culled and decreased milk yields in the first and second lactations (Robison et al., 1988; DeNise et al., 1989).

Nutrition of the newborn calf to weaning

After birth, calves are fed high-quality colostrum to provide passive immunity and this is done as soon as possible preferably within 3 h because of a rapid decrease of intestinal absorption ability of colostrum Ig with only 50% absorption by 9 h (Akins, 2016). Calves are fed colostrum at 12% to 15% of body weight, usually, 3.8 L assuming a 40-kg calf. High-quality colostrum contains at least 50 g Ig/L, free from blood, dirt, and from healthy cows.

The primary goal for calf growth is to double the weight by the time of weaning. The ADG would vary from 0.75 or 1 kg if the calf is weaned at 6 or 8 weeks respectively. To achieve this goal, nutritional strategies that combine liquid feeding using milk replacer as a protein and fat source while solid feeding or calf starter are fed to provide highly fermentable carbohydrates (starch and sugars). This type of feeding is considered to ensure good development of the reticulo-rumen as well as the growth of ruminal papillae for absorption of VFA. Butyric acid is the most important VFA used by ruminal epithelial tissues for papillae growth and subsequent absorption of VFA and ammonia.

Although feeding forages to heifers stimulate ruminal contractions and increase ruminal muscular development, forages are mostly needed after weaning because they may restrict starter intake and slows down the ruminal papillae development (Akins, 2016). Two feeding programs for calves have been used in North America: the conventional restricted program and the accelerated feeding program (Drackley, 2008).

The conventional restricted feeding program aims at providing a liquid feed at approximately 8% and 10% BW, whereas the intensive feeding offers liquid feed at 16% to 20% BW (Akins, 2016). The protein content of the conventional restricted feeding program based on milk replacer is of 20 to 22% to maximize lean tissue growth, and between 15% and 20% fat, whereas the protein content of accelerated feeding program would range between 26% and 28%, and fat content similar to the conventional feeding program (Drackley, 2008). Increasing fat content in milk replacer would increase ADG but decreases starter intake (Hill et al., 2006). Under thermoneutral conditions, the milk replacer containing low fat would favor lean tissue growth and increased starter intake (Drackley, 2008).

The two feeding programs described above provide different growth results for calves. Calves fed with the conventional program have increased calf starter intake at an earlier age than calves fed with the intensive feeding program. However, those calves on conventional feeding programs have lower energy and protein intakes from liquid feed, resulting in a lower growth rate during the first 2 weeks of life (Akins, 2016).

Calves on intensive feeding programs have greater daily gains from birth to weaning but the cost associated with this type of feeding program is higher compared to the conventional feeding program. Calves fed with the intensive program are bred and calve 15 to 30 days earlier and have been shown to have increased milk yield at first lactation (Drackley, 2008). Apart from feeding milk replacer, pasteurized whole or waste milk is another economical option for large dairies having enough waste milk to feed calves (Akins, 2016).

When an intensive feeding program uses whole milk instead of using milk replacer, it would further improve milk yield at first lactation (Akins, 2008). This finding is probably due to increased mammary fat pad mass having a paracrine and endocrine effect on mammary gland development (Moallem et al., 2010).

As soon as the calf starts to consume dry feed, there is an increase in ruminal microbial population and the absorption capacity of nutrients (Heinrichs and Lesmeister, 2005). Consuming the starter enables the calf to improve her capacity to use end products of the intake rich in easily fermentable ingredients (Drackley, 2008). The good calf starter provides a high rate of microbial protein synthesis and fermentation products as well as some bypass protein and starch that are digested in the small intestine.

Nutrition from weaning to breeding

According to Drackley (2008), calves are maintained on the starter until they are weaned at around 10 weeks of age when they are transitioned to a grower diet of lower protein content. After weaning, diets are formulated to allow heifers to reach the breeding age as soon as possible without having a higher fat deposition. Heifers should have the **BW** of 55% of mature BW at the time of breeding at 13 months of age and calve at 82% mature BW. The target age at first calving is at 22.5 months with the post-partum BW of 526 kg. The ADG for post-weaned heifers is between 0.8 at 0.9 kg/d provided that metabolizable protein (**MP**) supply is adequate as predicted by NRC or Cornell Net Carbohydrate and Protein System models (Akins, 2016).

From 3 to 9 mo of age, the mammary gland grows very fast and follows allometric growth and can be affected by nutrition practices during this growth period. If excess energy is fed, it results in a decreased proliferation of epithelial tissue and deposition of additional adipose tissue in the mammary gland with reduced milk production later in life (Akins, 2016). During the breeding period, heifers should be fed diets that provide adequate MP to favor lean tissue growth. When feeding heifers for faster growth, it is important to monitor the body condition to avoid excess adipose deposition.

Strategies on restricting growth rates during the prepubertal period have been investigated and have shown improvements in milk production compared with ad-libitum prepubertal feeding. Lammers et al. (1999) fed prepubertal heifers the same diets differing in amounts to result in gains of 0.7 kg and 1 kg per day. Heifers with 0.7 kg of ADG had 7% greater milk production than those with 1 kg of ADG, and this was probably due to different mammary development. There was no difference in age or weight at first calving because heifers fed to gain 0.7 kg/d had considerable compensatory growth.

In addition to additional skeletal and mammary growth during the prepubertal growth, the rumen continues to increase in size and microbial populations which allows the heifer to consume high-forage and cheaper diets because of ruminal volume and increased retention time of slowly digestible fiber (Akins, 2016). During the post-weaning period, forages are slowly increased in the diet to avoid a decrease of concentrate intake and weight gain.

According to Akins (2016), silages should not be fed to heifers until 3 mo of age due to a decreased DMI, possible mold contamination and poor ruminal use of highly degradable protein or non-protein nitrogen by young heifers. However, Drackley (2008) suggested that heifers can thrive when fed corn silage or alfalfa silage from a relatively early age especially when fed a total mixed ration (**TMR**). Results from a study by Denis et al. (1995), showed that heifers of approximately 4 mo of age fed grass hay had increased growth performance compared with heifers fed grass baleage because of increased DMI of the grass hay diet.

The common practice is to start to move heifers onto a TMR in the early grower phase by mixing the starter grain with small amounts of the high-group lactation TMR in DM ratios ranging from as little as 9:1 to as much as 4:1. When heifers are adapted to this regimen for several weeks, they can easily move to a TMR containing large amounts of silages without any negative effects.

At breeding time, heifers should have an adequate size for carrying a calf until birth and to minimize later negative effects (dystocia and low milk production) when heifers calve earlier than 22 mo of age. Later breeding can delay conception rate as well as the start of lactation. The general recommendation is to breed heifers when they are 55-60% of mature BW. Heifers should be prescreened at 12 mo of age for BW to decide whether they are ready for breeding. Heifers weighing 390 kg or greater at 12 mo are eligible for breeding, but this goal depends on the mature BW and the percentage of mature BW at breeding (Akins, 2016).

Nutrition from breeding to calving

After breeding, heifers should maintain adequate growth rates while minimizing excess body conditions gains. When heifers reach maturity, the rate of lean tissue deposition decreases while adipose deposition rate increases (Akins, 2016). If not properly monitored, heifers can quickly become over-conditioned even when fed a moderate-quality forage ad libitum or when heifers are fed diets high in energy content without a proper restriction.

During the gestation period, excess adipose tissue results in negative effects on heifers (metabolic disorders, dystocia, and low milk production) as they transition to lactation. To monitor the condition of heifers, body condition scoring should be consistently used to target 3.5 as the desired body condition at calving.

Nutritional strategies for optimum heifer development

Most of the nutritional strategies for growing heifers focus on maintaining an adequate rate of gain to reach the desired BW at breeding and first calving with the least amount of feed and cost inputs (Akins, 2016). An excellent reproductive program results in heifers that are bred at the correct age and weight with a low service and conception rate. A poor reproductive program results in a high service rate and a low conception rate with increased calving age, and additional days on feed, which significantly affect production costs.

Feed efficiency has attracted the interest of producers and researchers to decrease feed inputs and manure output while minimizing the risks of over conditioning. In this regard, two strategies have been investigated: limit-feeding heifers to only the energy amount needed and restricted intake which improves feed efficiency helps to control the body condition. The second option is for bred heifers which are fed forages with lower nutritive values and higher NDF as

those heifers have lower requirements for energy and protein compared with lactating cows (Akins, 2016). The benefits of feeding high fiber diets to heifers are to reduce ad libitum feed intake because heifers have an intake limit of approximately 1% of BW as NDF (Akins, 2016).

Limit feeding

Limit-feeding is a strategy that aims to meet nutrient requirements of growing heifers by feeding a diet with greater nutrient density but at lower feed intake. This feeding strategy helps to control heifer over conditioning, improve feed efficiency by reducing feed intake with similar weight gains and decreased fecal output. Limit-fed diets provide a daily amount of nutrients required by growing heifers for maintenance and growth. In contrast, to limit feeding, the ad libitum feeding system provides a less nutrient-dense diet and heifers can eat as much as possible to satisfy their gut fill (Akins, 2016). However, the ad libitum feeding system can lead to reduced feed efficiency and body over conditioning if the diet is not well balanced for lower energy content.

In a study by Zanton and Heinrichs (2007) on prepubertal heifers, a high forage diet (75% forage) was compared to a limit-fed high concentrate diet (75% concentrate) for 35 weeks. Both diets were formulated to provide similar nutrients amounts. Results showed similar weight gains, frame growth and improved feed efficiency for limit-fed diet. However, heifers limit-fed had greater paunch girth due to fat deposition.

The limit-feeding strategy requires additional management to ensure that heifers are fed correct feed amounts to prevent overfeeding or underfeeding. It is necessary to use a mixer wagon with a weigh scale and conduct regular nutrient analyses of feeds to adjust ratios of as-fed ingredients because changes in DM alter the amount of nutrients supplied. Limit fed heifers exhibit aggressive feeding behavior at the beginning as most of the feed is consumed within 1 or 2 h.

Feeds should be pushed up within 1 h to prevent heifers struggling to reach feed which could lead to increased shoulder abrasion and inner hoof wear especially on the front hoofs caused by pushing forward to reach the feed.

Adequate bunk space is highly important to make sure all heifers can have access to feed at the same time. Boss heifers may dominate submissive ones if there is inadequate feed bunk space and may result in lower intakes as well as insufficient weight gains.

Management aspects of implementing limit-feeding in dairy heifers

Group and weigh heifers often

According to Zanton and Heinrichs (2008), regular control of heifer's weight is the best management tool in limit-fed heifers since inappropriate diet restriction can cause rapid weight gains or lower gains than targeted. Heifers should be weighed once per month or less as long as the body condition is monitored. When heifers are grouped in the same facility, heifers beyond 4 mo of age should be grouped with other heifers as close to the same age as possible and in groups less than 90 kg of weight variation within the group. Heifers in a post-breeding period should be grouped with a maximum weight variation of 136 kg between animals.

Feed bunk space

Zanton and Heinrichs (2008), recommend the feed bunk size in a limit-feeding system to range between 35.5 to 60.9 cm from 4 mo to the pre-calving age of 22 mo of age and heifers should have access to feed at the same time. When the feeding space is limited, three strategies can be used: the first strategy is to group animals having a similar BW. The second strategy is to limit free motion at the feed bunk by using headlocks or closely placed divider posts. The last strategy is to feed twice daily but at close intervals. One example given for this strategy is to feed two-

thirds of the daily allotment at 7 a.m. and the remaining third at 9 a.m., to allow larger animals to eat more freely at the early feeding and submissive animals at the second feeding. In a precision feeding, it is imperative to choose suitable beddings because heifers will readily consume edible bedding which will affect the benefits obtained by limit-feeding.

Nutritional requirements of heifers in a limit-feeding system

Protein requirements

According to Zanton and Heinrichs (2008), the protein requirements for pre-pubertal heifers range between 14 to 15% CP and is based on 2.15% BW DMI/d. For postpubertal heifers, the protein requirements range between 13 to 14% CP based on 1.65% BW DMI/d. Soluble protein should be maintained at least 30 to 35% in the rations. Total protein requirements are of equal importance as various protein fractions. Ruminal undegradable protein is of limited value for heifers beyond what is provided by common feedstuffs. It has been demonstrated that soluble and RDP are efficiently utilized by dairy heifers which allows for efficient rumen microbial protein production throughout the day despite limited feed intake.

Energy requirements

The energy requirements of the heifer largely depend on the size, growth rate and the environment. According to Zanton and Heinrichs (2008), two feeding strategies have been identified to meet the energy requirements of growing dairy heifers. The first strategy is to formulate diets at variable energy densities and fed ad-libitum to allow the heifer to select her energy consumption.

The second strategy is to formulate a diet generally fixed at a higher energy content and limit-fed to meet the requirements of the dairy heifer. Either feeding strategy should allow the

heifer to consume an energy intake that favors an average daily gain of 0.79 to 0.97 kg or 286.6 kcal of metabolizable energy per kg of metabolic body weight ($BW^{0.75}$).

Fiber (NDF and ADF) requirements

The traditional feeding system of dairy heifers was based on feeding high levels of fiber or low-quality forage to control dietary energy. The recommendations of the NRC (2001) level for fiber content in dairy heifer's diets are not clear. It was found that diets as low as 19% NDF are not detrimental to dairy heifer's health with regards to metabolic disorders or lameness (Zanton and Heinrichs, 2016).

According to Zanton and Heinrichs (2016), the fiber levels in heifer's diets are expected to provide an amount of excess of requirements for adequate rumen function and should be at 60 or 70% of voluntary DMI because of limitations of gut fill. However, feeding a high-forage diet containing higher NDF and a lower energy content is used by producers to control weight gain and prevent excess body conditions of heifers. According to Hoffman and Kester (2012), heifers are only able to consume 1% of BW of NDF daily and this can be used to formulate diets to control intakes and weight gain, especially for bred heifers.

Conclusion

Dairy producers should pay close attention to heifer development to ensure proper growth and future lactation performance. The main objective for heifer production is to raise a heifer susceptible to calve between 22 and 24 months of age while minimizing feed costs, nutrient excretion and potentially improving subsequent milk production. Heifers calving before 22 months result in lower first lactation milk yield, where calving after 24 months results in excess days on feed and cost of heifer production.

While limit-feeding is becoming common practice to properly raise dairy heifers, including cheaper alternative feed sources such as agro-industrial byproducts in diets of growing heifers would be another strategy to minimize feed cost while improving animal performance. Based on its nutrient composition, WBG would be an ideal byproduct that can be used to replace soybean and corn-based concentrates in dairy heifers' diets. However, proper handling techniques needs to be used to improve the shelf-life of WBG at farm level to avoid spoilage caused by mold and yeast development.

CHAPTER II

Effect of storage of wet brewer's grains treated with salt or a commercially available preservative on the prevention of spoilage, in vitro and in situ dry matter digestibility and intestinal protein digestibility.

Abstract

Two experiments aimed to evaluate the effect of storage of wet brewer's grains (**WBG**) treated with salt or a commercial preservative (**PRES**) on 1) yeast and mold growth, and (**IVDMD**), and 2) in situ dry matter, and protein digestibility. In Experiment 1, control (0%), PRES (0.05%, 0.10%, 0.15%); or salt (1.4%, 2.6%, 3.8%) treatments were allocated randomly in duplicate to 14 plastic tubs containing of 48.5 kg of fresh WBG. The tubs were left inside a room with temperature varying from 12.8 to 14.4°C for 28 d. Samples were collected every 2 d and analyzed for yeast and mold concentrations. Subsamples collected on d 0, 7, 14, 21 and 28 were subjected to IVDMD in a batch culture fermenter. In Exp. 2, 3 cannulated cows were used twice in a replicated 3×3 Latin square to determine the DM, NDF, ADF and protein digestibility of WBG preserved for 1 wk with 0% or 0.10% PRES or 2.6% salt. In Experiment 1, WBG treated with PRES had the least yeast counts. The WBG treated with salt (3.8%) and PRES (0.15%) had the least mold counts. Greater IVDMD was observed in WBG treated with salt. In Experiment 2, treatment with 2.6% salt resulted in greater in situ DM and protein digestibility. Based on these data salt and PRES prevent spoilage of WBG, as indicated by yeast and mold counts, but salt has an advantage of improving DM and protein digestibility.

INTRODUCTION

Brewer's grains are residues of the brewery industry, which uses mostly malted barley to produce beer, leaving behind a protein-rich residue. Brewer's grains have been used in dairy cattle feeding and their nutrient contents, except for starch, are greater than barley. (Chiou et al., 1998; Mussatto et al., 2006).

Brewer's grains are a good source of **NDF** (Mertens, 1997) and **RUP** with a high content of methionine and lysine (Clark et al., 1987). Because of the moisture content of wet brewer's grains (**WBG**; 65-75%), improper storage often leads to large loss of DM and nutrients due to different biological activities of microorganisms responsible for spoilage (Lowes et al., 2000; Moriel et al., 2015), or due to temperature and storage time (Wang et al., 2014).

Several studies have been conducted to improve the preservation of WBG using different microbial inoculants (Lilly et al., 1980; Schneider et al., 1995) and commercial preservatives (Marston et al., 2009). However, little information is available on preventing spoilage of WBG by treatment with salt and how it compares with other inoculants. Cai et al., (1997) reported that adding 40 g of NaCl/kg of wet sorghum silage at ensiling reduced DM losses from 14 to 7%. Shockey and Borger (1991) observed a reduction in the number of *Clostridium* bacteria when 4g of NaCl/100 g was added to alfalfa silage. Moreover, McLaughlin et al. (2002) reported that salt-treated silage is likely more digestible than untreated silage and had less DM loss.

The objective of this study was to evaluate and compare the effect of storage of WBG with salt and a preservative (**PRES,** Silo-King GPX, Agri-King Inc., Fulton IL) on prevention of spoilage (as assessed by yeast and mold growth) and assess the effect on IVDMD, and in situ DM, NDF, ADF and intestinal protein digestibility. Salt inhibits sensitive aerobic bacteria either by dehydration, plasmolysis, or chloride toxicity (Taormina, 2010). We hypothesized that treating

WBG with PRES or salt would reduce yeast and mold growth while improving nutrient digestibility compared to the control.

MATERIAL AND METHODS

Experiment 1. Evaluation of treatment on Yeast and Mold growth and on IVDMD in WBG.

Feeds and experimental design.

Fresh WBG was collected from a local brewer (Bad Lab brewery, Somersworth, NH) and brought to the Fairchild Dairy Research Center at the University of New Hampshire (15.9 km). Initial WBG samples for Exp. 1 and 2 were sent to Analab (Fulton, IL) for analysis of ADF (method 973.18); NDF (method 2002.04); CP (method 990.03); starch (enzymatic method using glucose Trinder); crude fat (method 920.39); ash (method 942.05); Ca, P, Mg, and K (method 985.01) according to AOAC International methods (AOAC International,1999). Nutrient composition is presented in Table 1. Wet brewer's grains (48.5 kg) were transferred into 14 plastic tubs (cylinder shape: 42cm height to 42 cm diameter). Treatments were added to the WBG to achieve the following concentrations PRES: (0.05%, 0.10%, and 0.15%), salt: (1.4%, 2.6%, and 3.8%), or control: 0%. Treatments were assigned randomly in duplicate to the tubs and mixed. Instead of placing treatment only on the top of the tub, we mixed it throughout because we wanted to evaluate treatment effects in vitro and in situ (Exp. 2). The WBG was mixed with treatment using a shovel to mimic adding it throughout the pile, which would be accomplished commercially using a mixer wagon. The salt treatment amounts were based on previous studies conducted by Cai et al. (1997) and Shockey and Borger (1991) who added 4% salt to silages. The preservative treatment amounts were based on previous research in our laboratory using WBG where 0.05 and 0.10% were used (Marston et al., 2009). Anderson et al. (2015) added this PRES (0.10%) to distiller's wet grains with solubles (66.7%) mixed with 33.3% corn stalks. In the current study, the

first 2 amounts were similar to the experiment conducted by Marston et al. (2009) and Anderson et al. (2015). This experiment also used a greater concentration (0.15%). Plastic tubs containing treatments were left inside a room with temperature varying from 12.8 to 14.4°C for 28 d to evaluate treatment effect on the growth of yeast and molds.

The PRES (Silo King GPX, Agri-King Inc., Fulton, IL) is a dry, granular, free-flowing product that contains lactic acid producing bacteria *Lactobacillus plantarum*, *Enterococcus faecium*, and *Pediococcus pentosaceus* and fermentation extracts from *Aspergillus oryzae*, *Trichoderma longibrachiatum* and *Bacillus subtilis*. It also contains the preservative and anti-oxidant butylated hydroxytoluene (BHT), as well as anti-fungal agents such as potassium sorbate, sodium benzoate, propionic acid, acetic acid, benzoic acids, and sorbic acids. Moreover, the PRES contains monosodium phosphate which acts as a nutrient and acidulant, while sodium silico aluminate acts as a moisture scavenger (Marston et al., 2009).

Sampling for Yeast and Mold evaluation.

Two samples of WBG of approximately 300 g each were collected from each treatment (one sample per plastic tub) every 2 d for 28 d total. All samples were collected 20 cm below the surface to avoid the top layer. Sampling spots were always different from previous ones. Samples were collected from the center to different corners of the tubs to avoid sampling in the same spot consecutively. After removal, the hole in the WBG was carefully collapsed. Samples were then refrigerated at 4°C and sent twice a week to ANALAB (Division of Agri-King, Fulton, IL) for DM (AOAC, 1999, method 935.29), yeast and molds analysis (AOAC 1999, method 997.02), using culture plates of dry medium supplemented with antibiotics, dye to enhance visualization of growth, and cold H2O-soluble gelling agent. Undiluted or diluted suspensions are added to plates at a rate of 1 mL/plate. The suspension is spread over a 30 cm^2 growth area. Gelling agent is

allowed to solidify, plates are incubated, and yeasts and molds are counted and reported as cfu. Because this was a preservation study under aerobic conditions to mimic feeding brewer's grains on the farm, the pH and fermentation end-products were not measured, and the plastic tubs were not sealed.

Sampling for IVDMD of Brewer's Grains.

The IVDMD method was based on Ankom Technology Method 3 (Ankom Technology, Macedon, NY). Briefly, 2 samples of approximately 500 g were collected per treatment (one sample per plastic tub) on d 0, 7, 14, 21 and 28 and stored at 4°C. Samples of WBG were weighed and then dried in a forced-air oven (Binder, Bohemia, NY) at 55°C for 48 h to calculate the DM. Samples were then ground to pass through a 1mm screen using a Wiley mill (Model 3, Arthur H. Thomas Scientific, Swedesboro, NJ). A total of 5 g of ground sample from each treatment was weighed in duplicate into an Ankom 57 filter bag with a pore size of 50µm (Ankom Technology) and heat sealed. Two liters of ruminal fluid including and 500 g of the fibrous mat from the rumen were collected 4 h after morning feeding from a cannulated dairy cow maintained on a TMR (Table 2).

After collection with a vacuum pump into a pre-warmed thermos, the ruminal fluid was transported to the laboratory and blended under CO_2 for 30 s and strained through 4 layers of cheesecloth into a preheated flask. A buffer solution (1,600 mL; pH adjusted to 6.8) was prepared in advance (Ankom Technology, 2017) and prewarmed in digestion jars at 39°C in a Daisy Incubator (Ankom Technology) for at least 20 min before adding 400 mL of rumen fluid from the preheated flask.

Duplicate filter bags containing the dried and ground samples of WBG (28 samples) and 2 blank bags (to correct any feed loss) were evenly placed (14 bags on each side of flask divider)

into 4 digestion jars containing a mixture of buffer solution and ruminal fluid (4:1 ratio) for 48 h at 39.5°C with a constant rotation of the jars.

At the end of incubation, all bags were removed, rinsed with tap water until run-off was clear. Bags were then dried in a forced-air oven (Binder) at 55°C for 48 h, after which they were weighed to determine the DM digestibility.

Statistical analysis.

Yeast and mold concentrations were reported as cfu/g of wet sample and were logarithmically transformed and analyzed as a completely randomized design using RStudio (RStudio v. 3.4.1, Inc., Boston, MA) according to the following model:

$Y_{ijk} = \mu + \tau_i + D_j + \tau D_{ij} + \varepsilon_{ijk}$, where Y_{ijk} is the dependent continuous variable, μ is the overall, mean, τ_i is the fixed effect of ith treatment (1, 2, 3, 4, 5, 6, 7), D_j is the random effect of jth days of storage (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 28), τD_{ij} is the effect of day and treatment interaction and ε_{ijk} is the residual error.

Contrasts were used to compare treatments means (salt vs PRES) and determine linear, and quadratic effects of increasing levels of salt or PRES on dependent variables. Significance was declared at $P \leq 0.05$ while tendency was declared at $0.05 < P \leq 0.10$. The ANOVA on the DM content and IVDMD of WBG was performed using the same model as described above.

Experiment 2: Evaluation of treatment on In Situ DM, NDF, ADF and Intestinal Protein Digestibility of WBG.

Feeds and experimental design.

Fresh WBG (see Table 1 for nutrient composition of batch 2) were obtained from a local farm (Stuart Farm, Stratham, NH) and transported to the Fairchild Dairy Research Center (17.6

km). Based on Exp. 1 results, 3 treatments (control, 2.6% salt, and 0.10% PRES) were prepared to be used for Exp. 2. In Exp. 1, PRES at the rate of 0.10% had the least yeast concentration, and salt at 2.6% reduced mold growth. Although the least mold growth observed for the 3.8% salt treatment, we did not choose that treatment for evaluation in Exp. 2 due to the amount of salt used. Nutrient compositions of WBG used in experiment were determined using the same procedures as described in Exp. 1.

Tubs were filled with wet brewer's grains and weighed on a platform scale (Cardinal, Northeast scale, Co. Inc., Hooksett, NH) to get the desired amount (48.5 kg). Treatments were then prepared and assigned randomly to 3 tubs. Treatments were evenly spread on the top and a shovel was used to completely mix the content for at least five minutes from top to bottom and side to side of the tubs.

Treatments were then left inside a room for 1 wk after which a duplicate of approximately 3-kg sample from each treatment, was collected (20 cm below the surface to avoid the spoiled top layer) and dried in a forced-air oven (Binder) for 48 h. One week was chosen to evaluate the effect of treatment over a typical feed-out period for WBG in the northeast United States.

Dried samples were ground to pass through a 1-mm screen using a Wiley mill (Model 3, Thomas Scientific). Five grams of each ground treatment sample was weighed into a 5 × 10 cm Dacron bags (Ankom R 510, pore size 50 µm, Ankom Technology) in duplicate and heat sealed. Dacron bags had pore sizes of 50 µm which is 20 times smaller than a feed particle size of 1 mm. Consequently, feed particles would likely not escape from the bag, and any losses could be easily corrected using blank Dacron bags (Payne et al., 1972; Orskov et al., 1980).

Animals and feeding.

All procedures involving animals were reviewed and approved by the University of New Hampshire Institutional Animal Care and Use Committee (Protocol #170603). Three multiparous lactating Holstein cows fitted with rumen cannula, with BW of 814 ± 54.2 kg, were used twice in replicated 3 × 3 Latin square. Three periods of 48 h each were repeated twice (6 periods in total), each cow receiving a different treatment in each period. Sample bags were inserted in the rumen in reverse order as follows: 48 h, 24 h, 12 h, 8 h, 4 h, 2 h, and they were all removed at the 0 h. Cows were housed in a tiestall barn and fed individually using wooden boxes (90 cm × 90 cm × 90 cm) to control DMI. The 3 lactating cows were maintained on a TMR (Table 3). Cows were fed twice a day after milking at 0700 and 1600 h. Orts were collected and recorded before p.m. feeding to allow the calculation of the DMI for each cow.

Experimental procedure for In Situ DM, NDF, ADF and Protein Digestibility.

Dacron bags were prepared in duplicate for each cow and treatment for 0, 2, 4, 8, 12, 24 and 48 h. Six bags were prepared for the 12-h incubation period for each treatment to ensure enough residue was left for the intestinal protein digestibility procedure.

Before incubation in the rumen, bags were soaked in warm water (39°C) for 20 min. The 0-h samples were soaked in the same way but were not placed in the rumen. Within each cow, sample bags were placed in a large nylon mesh bag (36 × 42 cm) and submerged beneath the particulate mat layer in the rumen. Bags were inserted in reverse order and were all removed at the same time, submerged in a 10-L bucket of water, gently agitated and rinsed in cold tap water until run-off was totally clear. Bags were then frozen (-20°C) until the end of the experiment. Sample bags were thawed, rinsed again individually and dried in a forced-air oven (Binder) at 55°C for 48 h, and weighed to determine DM digestibility of WBG.

Neutral Detergent Fiber and ADF digestibility were determined according to Van Soest et al. (1991), using an Ankom^{2000} fiber analyzer (Method 13 and 12 respectively, Ankom Technology Corp). For NDF analysis, sodium sulfite and α- amylase was not used due to the low starch content of WBG. Dried residues (0.5 g) from in situ DM digestibility at time point 2, 4, 8, 24 and 48 h were weighed in duplicate into Ankom F57 filter bags, heat sealed and analyzed for the fiber analyzer for NDF first and ADF second. Washout-value (0 h) data were not available for NDF and ADF analyses because the 0- and 12-h time point in situ DM residues were used for the intestinal protein degradability analysis.

Intestinal digestibility of RUP was determined using the modified 3-step procedure (Gargallo et al., 2006) with further modifications as described by Lawrence and Anderson (2018). Residues from the 0- and 12- h incubation time point were composited for each treatment by cow. One gram of dried sample residue from the 12-h time point was weighed in duplicate into 5 × 10 cm Dacron bags (Ankom R510, pore size 50μm (Ankom Technology) and heat sealed. In each of the 3 incubation bottles of the Daisy^{II} incubator (Ankom Technology), 18 samples from each treatment and 2 blanks (to correct any feed particle loss) were incubated using 2 L of pre-warmed 0.1 N HCl solution (pH 1.9) containing 1 g/L of pepsin (Cas. #9C01-75-6, Acros, NJ).

The bags were incubated with constant rotation at 39°C for 1 h. After the incubation procedure, bottles were drained, and samples were rinsed with cold tap water until runoff was clear. The bags were then placed back into the same incubation bottles with 2L of pre-warmed pancreatin solution (pH: 7.2) that was prepared in advance (Gargallo et al., 2006) and incubated with constant rotation at 39°C for 24 h. After removal, bags were rinsed on cold tap water until runoff was clear and dried at 55°C for 48 h in an air-forced oven (Binder). The residue was then composited for each cow and period and sent to ANALAB (Division of Agri-King) for protein

analysis (Soluble, RDP and intestinal digestible protein; AOAC International, 1999, method no.990.03).

Statistical analysis.

Data from in situ DM, NDF and ADF digestibility, and ruminal and intestinal protein digestibility of WBG treated either with salt, PRES (Silo-king GPX) or the control, were analyzed as a 3 × 3 Latin square using RStudio (RStudio v. 3.4.1, Inc.) according to the following model: $Y_{ijkl} = \mu + p_k + c_l + \tau_i + h_j + \tau h_{ij} + \varepsilon_{ijkl}$, in which: Y_{ijkl} = Dependent continuous variable, μ = Overall mean, p_k = period effect, c_l = cow effect, τ_i = Fixed effect of i_{th} treatments, h_j = random effect of jth hours of incubation, τh_{ij} = treatment by hour interaction, and ε_{ijkl} = residual error. The LSMEANS option was used to generate least square means of treatments. Tukey test was used to separate means and significance was declared at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.10$.

To estimate degradation parameters, data of DM digestibility were fitted to the equation of Orskov and McDonald (1979), where degradation parameters give an estimate of the soluble fraction (a), the degradable fraction (b), and the undegradable fraction (c). Those parameters are usually estimated by an iterative least square procedure, but because this procedure often gives unrealistic estimates for (a + b), which represents the maximum extent of degradation (Erasmus et al., 1990a, b), the hand-fit method proposed by Orskov (1982) was used. The effective degradability **(ED**) of DM was a measure of digestion in the rumen over time, while considering the rate at which it flowed from the rumen to the small intestine. The ED was calculated by using rumen outflow rates of 2, 5 and 8% (Van Soest et al. 1991). The following equations were used:

$Y(t) = a + b\ (1- e^{-ct}),\ t \geq 0,$

$ED = a + b \times c / (c + k),$

where Y(t) is the fraction disappearance at time t, a is soluble or rapidly degradable fraction, b is the insoluble but potentially degradable fraction, c is the degradation rate, t is the incubation time, ED is the effective degradability for response variables (%), and k is the outflow rate of passage (h^{-1}).

RESULTS AND DISCUSSION

Yeast and mold growth and in vitro DM digestibility.

The effect of treatment by time on DM content, and yeast, and mold counts, as well as IVDMD, is presented in Table 4. No treatment × time interaction existed for DM of WBG. Day had no effect on DM, but treatment did affect DM ($P = 0.03$), with the least DM being for 0.15 % PRES treatment. The DM content of salt treatments was greater than PRES treated WBG ($P < 0.0001$), which may be due to the greater amount of dry material added to the salt treatments compared to the PRES treatments. Dry matter of PRES-treated WBG DM was less than that of salt-treated WBG, which was not expected to change due to the content in organic acids (e.g. lactic acid from lactic acid producing bacteria) that would prevent dry matter loss (Lambert and Stratford, 1999). The PRES treatment had a linear effect ($P = 0.003$) of PRES on DM. As PRES increased, DM content decreased. There was no quadratic effect for PRES, nor were there any effects on DM content.

For yeast concentration (log cfu) in WBG, a treatment × day interaction existed ($P = 0.01$; Table 4 and Figure 1). Treatment affected log cfu yeast counts ($P = 0.003$). Salt did not affect yeast counts ($P = 0.36$ for linear and $P = 0.94$ for quadratic effect). As PRES was increased, there was a quadratic effect ($P = 0.001$) resulting in reduced yeast with the least count being for the 0.10% treatment. This was due to the different organic acids such as benzoic and sorbic acids having strong antifungal properties. These acids have been used to increase aerobic stability in silage

(Kleinschmit et al., 2005). The antimicrobial properties of those organic acids come from their ability to pass across the cell membrane in the undissociated form and release hydrogen in the cytoplasm. The reduction in cytoplasmic pH or the use of ATP to resist the pH decline and maintain homeostasis causes the cell to reduce or stop growing (Lambert and Stratford, 1999). The lack of salt impacting yeast count was probably due to the tolerance of yeasts to high concentrations of salt (Masui et al., 1979). Yeasts have a very high density of negative charges on the surface on their membrane lipid bilayers, and this is thought to be a necessary adaptation to counter the high cation concentration (Na+ on the outside and K+ on the inside; Russel, 1989).

There was a trend ($P = 0.06$) of treatment × time interaction for mold concentration (log cfu; Table 4 and Figure 2), with the 0% having greater mold concentration than either treatment over the experiment. Mold concentration in salt treated WBG was not different from that in PRES treated WBG ($P = 0.85$). The least mold concentration was observed in WBG treated with 3.8 % of salt, followed by WBG treated with 0.15 % PRES and WBG treated with 2.6 % salt.

The capacity of salt to inhibit mold growth is due to the hyperosmotic shock that causes shrinkage of the cytoplasmic volume, a process that is known as plasmolysis (Csonka, 1989). Moreover, the lessening of water activity has been viewed as the most likely cause for microbial growth inhibition by salt, whereby vital microbial and enzymatic processes are interrupted (Lawrence et al., 2003; Albarracin et al., 2011).

In vitro dry matter digestibility was affected by treatment × day ($P < 0.0001$; Table 4 and Figure 3). Treatment with salt improved IVDMD linearly ($P = 0.001$). Adding PRES did not improve IVDMD. The greater digestibility of WBG treated with salt is probably due to the capacity of salt to improve and soften the texture of feeds (Van Buren, 2006). Possibly the salt treatments went into solution and enhanced IVDMD through a potential rumen microbial effect. Similar results

were reported by McLaughlin et al. (2002), who observed a reduction of NDF content of salted silage because of a reduction in aerobic loss of DM. The lower DM digestibility of WBG treated with the PRES and the control may also be related to the loss of organic matter and availability of nutrients in stored WBG (Mills and Kung, 2002; Marston et al., 2009).

The effectiveness of PRES against both mold and yeast growth in WBG may be explained by its overall composition of different lactic acid-producing bacteria (lowering the pH) and antifungal agents in treated WBG (Marston et al., 2009). The capacity of salt to inhibit mold growth may be mainly explained by its properties of causing plasmolysis and being toxic to molds.

In situ DM, NDF, ADF digestibility and intestinal protein digestibility

In situ DM digestibility increased with time in all treatments; there was a treatment × hour effect ($P = 0.002$). Salt-treated WBG had greater in situ DM digestibility (Figure 4) compared to PRES-treated WBG and the control ($P < 0.0001$). In situ DM digestibility of the control and PRES treated WBG was not different.

The DM degradation parameters of WBG treated with salt or preservative exhibited differences for the soluble fraction (a), which was higher for salt-treated WBG ($P < 0.0001$) and for the potentially degradable fraction (a + b; $P < 0.0001$; Table 5**)**. Treatments did not differ for the insoluble but potentially degradable fraction (b). Our findings agree with those by Marston et al. (2009), who reported no treatment effect on the degradable fraction (b) of WBG when treated with 0.45 kg or 0.9 kg of PRES.

The degradation rate (c) was greater for salt-treated WBG ($P < 0.0001$) than for PRES treated WBG or control. This explains greater values of ED of the DM that were observed with salt treatment ($P < 0.0001$) considering the rumen digesta outflow rates (2, 5, and 8%; Table 5).

The degradation rate (c) was calculated at the 8-h time point when ruminal DM degradation is high (Orskov et al. 1980). Both in situ and in vitro experiments have shown that salt treatment improved DM digestibility of WBG.

Compared with IVDMD of WBG, the overall in situ DM digestibility was greater due to differences in physical incubation conditions and differences in microbial ability to degrade substrate in the early and late incubation times. Physical incubation conditions such as pressure exerted on bags by rumen contractions during incubation times and faster rates of rumen fluid flow through the bags (Lindberg and Knutsson, 1981; Dewhurst et al., 1995) in the in situ procedure, could result in larger losses of particles and degraded compounds from the bags.

Moreover, microbial ability to degrade substrates may be affected by many factors which could shock microbial inoculum in an in vitro procedure. Those factors are source of rumen inoculum, composition and nutrient availability of diets offered to animal donors, rumen sampling time, inoculum preparation, sustained anaerobic environment during inoculum preparation, composition of the buffer solution, relative proportions of inoculum and medium, and the pH during incubation, which has been reported to bias in vitro data, particularly at early incubation times (Mertens, 1993; Weiss, 1994; Mould et al., 2005).

In our experiment, in situ DM digestibility of WBG in the control at 48 h of incubation is slightly less than WBG digestibility values found by Armentano et al. (1986) using 72 h of incubation (66.7% vs 70%, respectively) and was less than what was reported by Marston et al. (2009), who found ruminal digestibility of nontreated WBG of about 82.9%. The differences in digestibility of WBG may probably be due to differences in nutrient composition of grain varieties and brewing technology used (Muthusamy, 2014).

In situ digestibility of NDF and ADF was not different among treatments (Figure 5 and 6 respectively). Regardless of treatment, WBG exhibited high NDF digestibility at the beginning of the fermentation in the rumen (2 h) and achieved a peak at 24 h of incubation and then declined. Greater digestibility of NDF at the beginning of fermentation indicates high NDF solubility of WBG and it is reported to be an important criterion for feed quality (Bal et al., 1997). According to Bartolome et al. (2002), brewer's spent grains are rich in hemicellulose (39%) especially in arabinoxylans, which are highly digestible. This may explain the greater digestibility of NDF in our experiment. The digestibility of ADF was lower in the first few hours (4 h) and slightly increased for the rest of the incubation time. Compared to NDF, ADF digestibility was lower because it is mainly made of poorly digestible cell walls such as cellulose and lignin (Van Soest, 1994).

Figure 7 illustrates the effect of different treatments on the digestibility of different fractions of protein content in WBG. Protein digestibility followed the same trend as the in-situ DM digestibility. Soluble, rumen degradable and intestinal digestible protein fractions were greater ($P < 0.0001$, $P = 0.002$ and $P = 0.008$, respectively) in salt treatments than in the control and in the PRES treatments.

In this experiment, the soluble protein fraction from the control was 11.3% of CP which is in the range reported by Westendorf et al. (2014) of 4.4 to 12.2% of CP. Average RDP for the control and WBG treated with PRES was 23.5% and 22.4% respectively. These were slightly less than RDP of 24.4 to 35.3% reported by Westendorf et al. (2014). Our findings agree with those of Seymour and Polan (1986) who reported that a significant portion of WBG protein is made of RUP.

Although WBG has low ruminal protein digestibility, treating WBG with salt increased ruminal and intestinal protein digestibility (34.8 and 62.0%, respectively) compared to the control and PRES. This was probably due to increased DM and NDF digestibility as well as improved ruminal conditions (Faccenda et al., 2017).

CONCLUSION

Preventing spoilage of WBG by inhibiting mold and yeast growth is very important to preserve feedstuff quality and increase its nutrient digestibility. Results from of the current research suggest that treatment of WBG with a preservative (Silo-King GPX; Agri-King) slows down growth of yeast and mold, whereas salt treatment inhibits mold development. After 48 h of incubation, in vitro and in situ DM digestibility were greater for salt treatments at levels of 3.8% and 2.6% (63.5% and 75.7%, respectively). Additionally, treating WBG with salt may improve the soluble, rumen degradable and intestinal protein digestibility. Neutral detergent fiber and ADF digestibility were not affected by preservative of salt treatment. In response to our hypothesis, the commercial preservative and salt inhibited the spoilage of WBG by reducing yeast and mold growth. More interestingly, salt treatment improved DM and intestinal protein digestibility of WBG. Future research should investigate the effect of treating WBG with salt on in vivo nutrient digestibility and animal performance.

Table 1: Nutrient composition of two batches of WBG used in Experiment 1 and 2

	Batch1[1]	Batch2[2]
Item	% DM	
DM	18.1	20.9
CP	34.9	33.4
NDF	48.3	47.7
ADF	20.1	21.9
Fat	10.8	10.8
Starch	1.9	2.22
Ash	4.9	3.67
Ca	0.22	0.21
P	0.56	0.39
Mg	0.17	0.15
K	0.07	0.17

[1]WBG used in Experiment 1 for evaluation of mold, yeast, and IVDMD.

[2]WBG used in Experiment 2 for in situ DM, NDF, ADF and protein digestibility.

Table 2: Ingredient and nutrient composition of diets fed to dairy cows used for in vitro dry matter digestibility (IVDMD).

Ingredient	**% DM of total diet**
Corn silage	44.8
Energy mix[1]	18.4
Protein mix[2]	14.7
Grass silage	12.6
Mineral mix[3]	3.5
Alfalfa hay	3.4
Blood meal	1.7
Rumen-protected fat[4]	0.9
Nutrients	**% DM**
CP	15.6
NDF	57.6
ADF	23.8
Starch	18.9
EE[5]	3.4
Ca	0.8
P	0.4
Mg	0.4
K	1.4
Na	0.4
Se (mg/kg)	0.3
Vit-E (IU/kg)	41.6

[1]Contained pellet mill molasses (6.3%), fine corn meal (57.9%), steam-flaked corn (19.1%) and whole beet pulp (16.7%).

[2]Contained 7.28% distillers; 69.14% soybean meal; 21.83% canola and 1.75% urea.

[3]Contained 16.18% Ca, 1.17% P, 5.8% Mg, Salt 12.2%, 12.5% Na, 0.13% Fe, 0.11% Zn, 0.07% Mn, 7.49% Cl, 252.3 mg/kg Cu, 20.2 mg/kg Co, 8.14 % Se, 2.7 mg/kg organic Se, 9.5 mg/kg I.

[4]Bergafat (99.9% fat), (Nutrilinx, LLC, Hardwick, VT)

[5]Ether extract, petroleum

Table 3: Ingredient and nutrient composition of diets fed to dairy cows used for in situ nutrient digestibility

Ingredients	**% DM of total diet**
Corn silage	45.5
Grass silage	27.7
Protein mix[1]	14.7
Energy mix[2]	9.3
Mineral mix[3]	2.7
Nutrients	**% DM**
CP	15.7
NDF	37.6
ADF	23.8
Starch	18.9
EE[4]	3.4
Ca	0.8
P	0.4
Mg	0.4
K	1.4
Na	0.4
Se (mg/kg)	0.3
Vit-E (IU/kg)	41.6

[1]Contained pellet mill molasses (5.0%), fine corn meal (45.8%), steam flaked corn (15.2%) and whole beet pulp (33.9 %).

[2]Contained 7.3% distillers; 69.1% soybean meal; 21.8% canola meal and 1.7% urea.

[3]Contained 16.2 % Ca, 1.2% P, 5.8 % Mg, Salt 12.2 %, 12.5 % Na, 0.13 % Fe, 0.11% Zn, 0.07 % Mn, 7.5 % Cl, 252.3 mg/kg Cu, 20.2 mg/kg Co, 8.1 % Se, 2.7 mg/kg organic Se, 9.5 mg/kg I.

[4]Ether extract, petroleum

Table 4: Comparison of treatment effects on DM, yeast, mold, and IVDMD in WBG over 28 d (Experiment 1)

	Treatments[1]								*P*-value	Contrast *P*-values	PRES		Salt	
Item	0%	0.05% PRES	0.10% PRES	0.15% PRES	1.4% S	2.6% S	3.8% S	SE	Trt × d	S:PRES	L	Q	L	Q
DM, %	17.0[a]	16.0[ab]	16.6[ab]	14.4[b]	16.8[a]	17.8[a]	17.3[a]	2.01	0.71	0.0001	0.003	0.3	0.43	0.84
Yeast, log (cfu/g)	8.21[a]	8.01[ab]	7.64[c]	7.90[bc]	8.16[ab]	8.16[ab]	8.12[ab]	0.07	0.01	0.001	0.36	0.001	0.36	0.94
Mold, Log (cfu/g)	5.63[a]	5.13[ab]	5.08[ab]	4.29[bc]	5.36[a]	4.83[abc]	4.21[c]	0.19	0.06	0.85	0.001	0.5	0.03	0.8
IVDMD, %	54.4[cd]	52.0[e]	54.9[c]	52.7[de]	58.2[b]	58.6[b]	63.2[a]	1.48	0.0001	0.0001	0.47	0.9	0.001	0.51

[a-e] Means within rows with unlike superscripts differ ($P < 0.05$)

[1]0% = WBG not treated with the commercial preservative nor with salt; 0.05% PRES = WBG treated with the commercial preservative at the rate of 0.05%; 0.10%PRES = WBG treated with the commercial preservative at a rate of 0.10%; 0.15% PRES = WBG treated with the commercial preservative at the rate of 0.15%; 1.4% S = WBG treated with salt at the rate of 1.4%; 2.6% S = WBG treated with salt at the rate of 2.6%; 3.8%S = WBG was treated with salt at a rate of 3.8%. Trt × d = treatment by day interaction. S:PRES = contrast between salt treatments and the preservative treatments. L = Linear effect. Q = Quadratic effect.

Table 5: DM degradation parameters and effective degradability (ED) of WBG (Experiment 2)

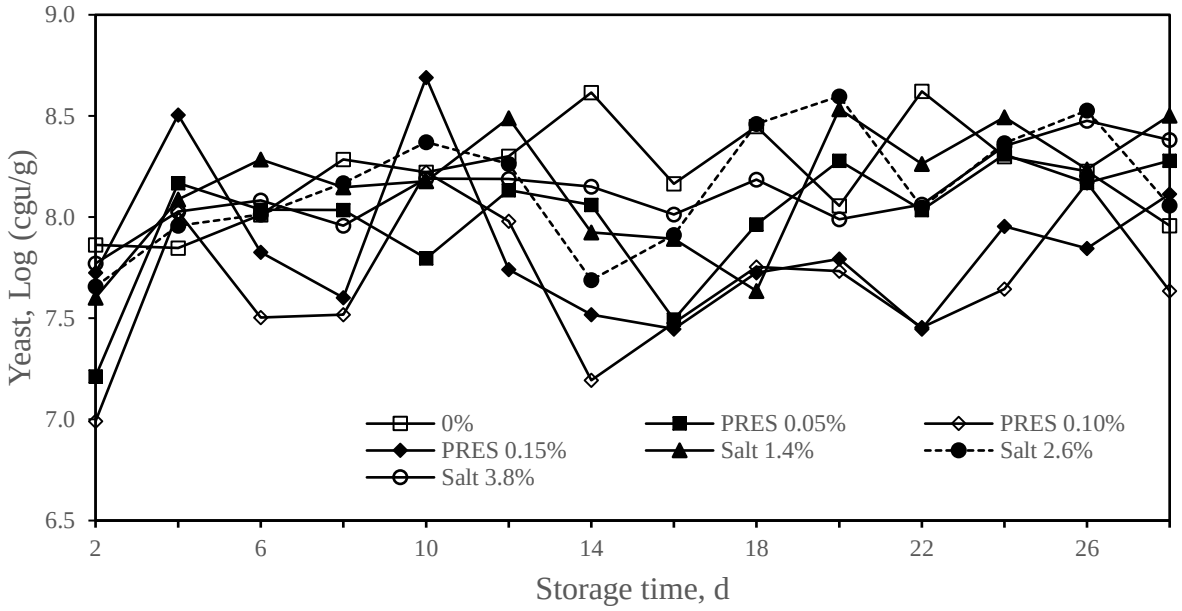
Treatments	Degradation parameters[1]				ED		
	a	b	a + b	c	k = 2%	k = 5%	k = 8%
0%	24.2[b]	41.3[a]	65.5[b]	0.052[b]	56.0[b]	47.3[b]	42.4[b]
PRES 0.10%	22.1[b]	42.0[a]	66.1[b]	0.046[b]	52.3[b]	43.5[b]	38.9[b]
Salt 2.6%	35.9[a]	39.7[a]	75.7[a]	0.078a	72.5[a]	66.5[a]	59.7[a]
SEM	1.5	1.9	1.3	0.006	2.6	2.5	2.1
P-value[2]	0.0001	0.55	0.0001	0.0001	0.0001	0.0001	0.0001

0%: WBG not treated with PRES nor with salt; PRES 0.10%: WBG treated with the commercial preservative at a rate of 0.10%; Salt2.6%: WBG treated with salt at a rate of 2.6%; SEM: Standard error of the mean.

[1] a: Washing losses, soluble or rapidly degradable; this value is the intercept of the degradation curve at 0 h (%); b: the insoluble but potentially fermentable (%), c: degradability rate (h^{-1}); a +b: potential degradability (%); k: outflow rate of WBG; ED: Effective degradability of DM.

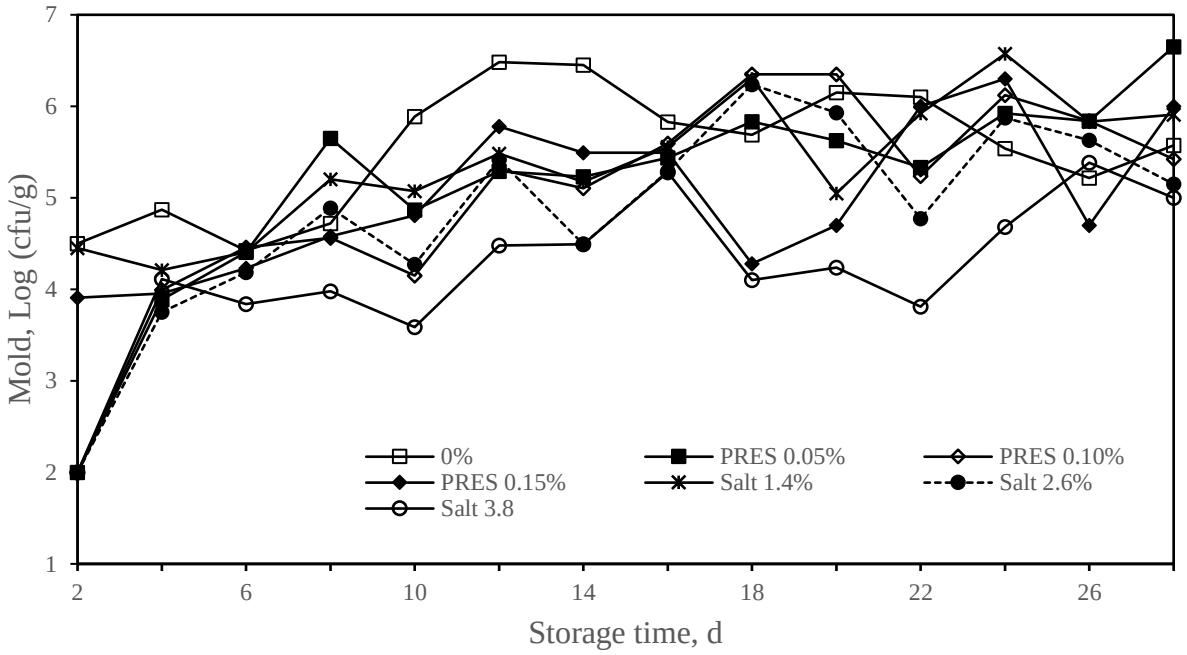
[2] Means within the same column with different superscripts (a, b, and c) differ ($P < 0.05$).

Figure 1. Effect of treatment of WBG with different levels of PRES and salt on yeast counts.



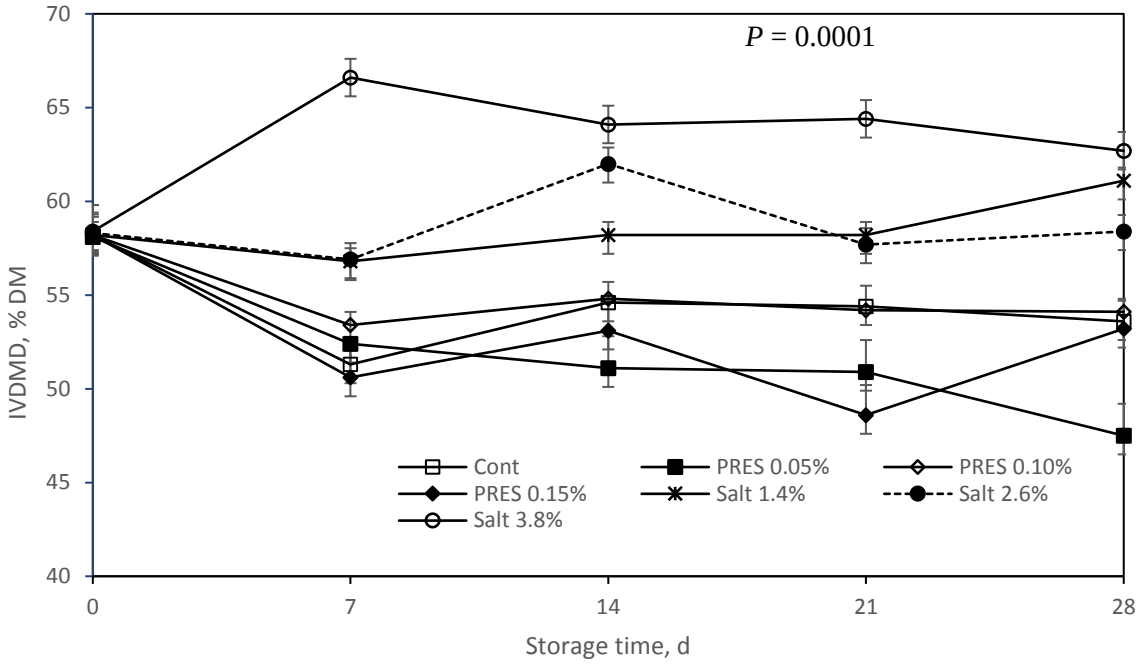
0% = WBG not treated with PRES nor with salt. PRES 0.05% = WBG treated with the commercial preservative at a rate of 0.05%. PRES 0.10% = WBG treated with the commercial preservative at a rate of 0.10%. PRES 0.15% = WBG treated with the commercial preservative at 0.15%. Salt 1.4 = WBG treated with salt at a rate of 1.4%. Salt 2.6% = WBG treated with salt at a rate of 2.6%. Salt 3.8% = WBG treated with salt at a rate of 3.8%. The largest standard error was 0.19 for PRES 0.15%.

Figure 2. Effect of treatment of WBG with different levels of PRES and salt on mold counts



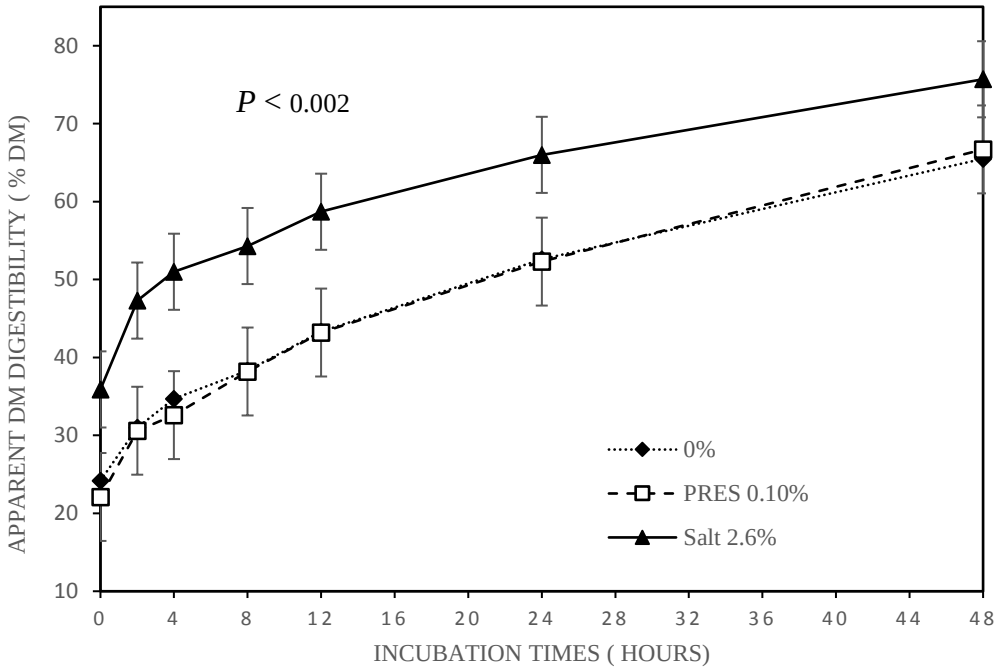
0% = WBG not treated with PRES nor with salt. PRES 0.05% = WBG treated with the commercial preservative at a rate of 0.05%. PRES 0.10% = WBG treated with the commercial preservative at a rate of 0.10%. PRES 0.15% = WBG treated with the commercial preservative at 0.15%. Salt 1.4 = WBG treated with salt at a rate of 1.4%. Salt 2.6% = WBG treated with salt at a rate of 2.6%. Salt 3.8% = WBG treated with salt at a rate of 3.8%. The largest standard error was 0.54 for PRES 0.10%.

Figure 3: Effect of treatment of WBG with different levels of PRES and salt on IVDMD.



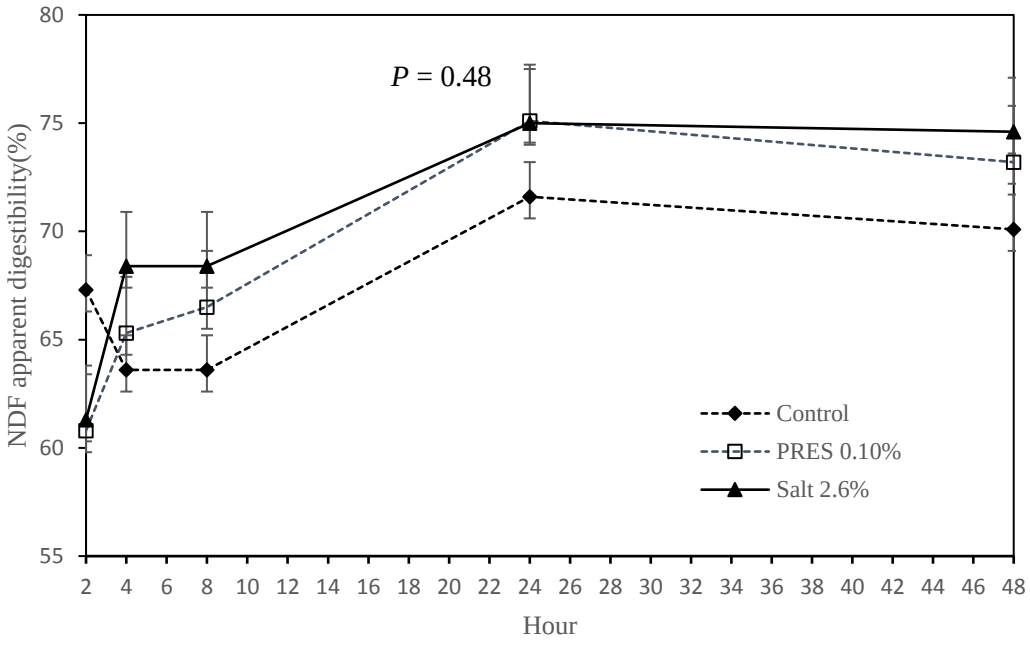
0% = WBG not treated with PRES nor with salt. PRES 0.05% = WBG treated with the commercial preservative at a rate of 0.05%. PRES 0.10% = WBG treated with the commercial preservative at a rate of 0.10%. PRES 0.15% = WBG treated with the commercial preservative at 0.15%. Salt 1.4 = WBG treated with salt at a rate of 1.4%. Salt 2.6% = WBG treated with salt at a rate of 2.6%. Salt 3.8% = WBG treated with salt at a rate of 3.8%. The largest standard error was 1.1 for Salt 2.6%.

Figure 4: In situ DM digestibility of brewer's grains treated with 0.10% PRES or 2.6% salt



0% = WBG not treated with PRES nor with salt; PRES 0.10% = WBG treated with the preservative at a rate of 0.10%; Salt 2.6% = WBG treated with Salt at a rate of 2.6%. In situ DM digestibility was measured on WBG residues from different incubation times (0, 2, 4, 8, 12, 24 and 48 h) in the rumen. The largest standard error was 1.05 for PRES 0.10%.

Figure 5: Effect of treatment of brewer's grains with 0.10% PRES or 2.6% salt on NDF digestibility

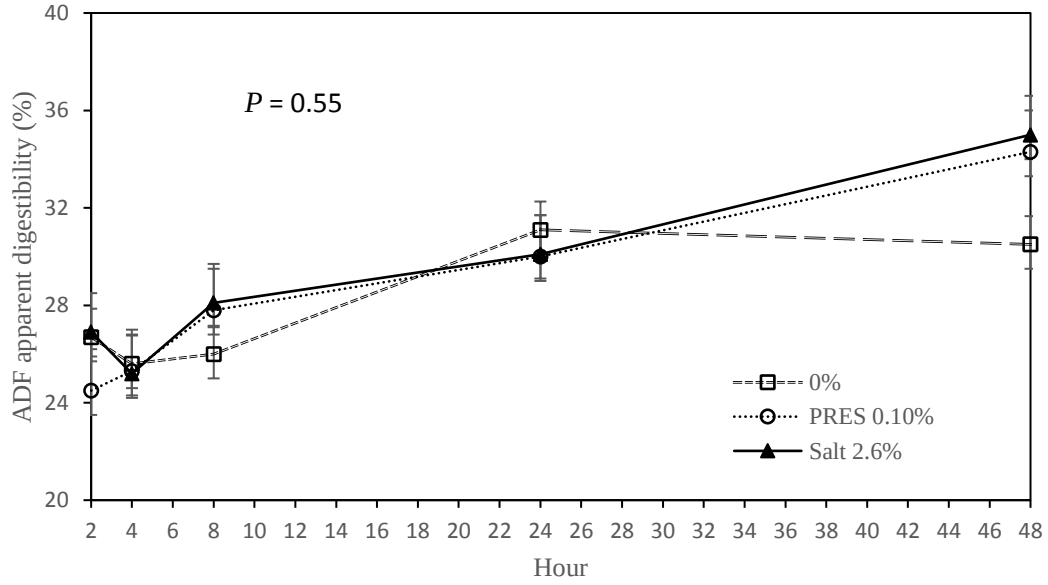


0% = WBG not treated with salt nor with a commercial preservative;

PRES 0.10% = WBG treated with the commercial preservative at a rate of 0.10%;

Salt 2.6% = WBG treated with salt at a rate of 2.6%. In situ NDF digestibility was measured on WBG residues from different incubation times (2, 4, 8, 24 and 48 h) in the rumen. The largest standard error was 0.82 for Salt 2.6%.

Figure 6: Effect of treatment of wet brewer's grains with 0.10% PRES or 2.6% salt on ADF digestibility

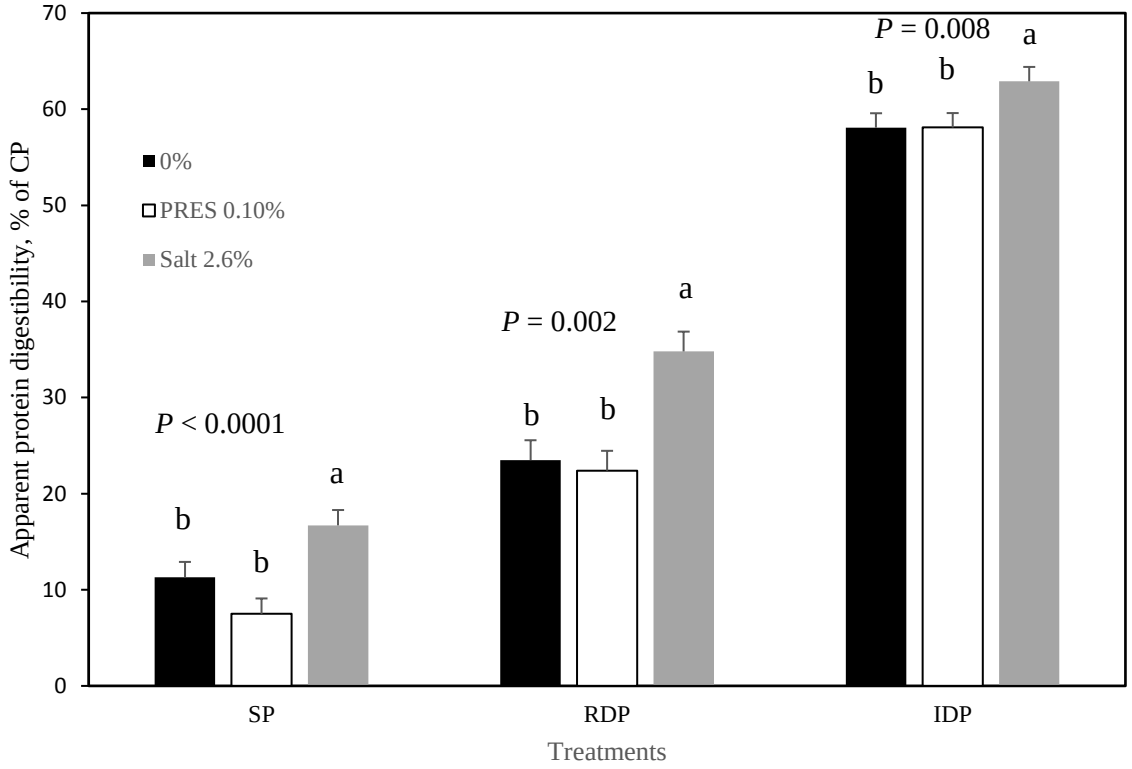


0% = WBG not treated with salt nor with PRES with commercial preservative;

PRES 0.10% = WBG treated with the commercial preservative at a rate of 0.10%;

Salt 2.6% = WBG treated with salt at a rate of 2.6%. In situ ADF was measured on WBG residues from different incubation times (2, 4, 8, 24 and 48 h) in the rumen. The largest standard error was 0.56 for Salt 2.6%.

Figure 7: Effect of treatment of wet brewer's grains with 0.10% PRES and 2.6% Salt on protein digestibility



SP = Soluble protein which is the instantly degradable protein fraction;

RDP = Rumen degradable protein that was measured from WBG residues from the 12 h time point of incubation in the rumen;

IDP= Intestinal digestible protein was measured on WBG residues from the 12 h time point of incubation in the rumen and were sequentially incubated in 0.1N HCl solution and pancreatin solution. Treatments with different superscripts differ ($P < 0.05$)

CHAPTER III

Growth performance and apparent total-tract nutrient digestibility of limit-fed diets containing wet brewer's grains to Holstein heifers

ABSTRACT

The objective of this study was to evaluate the growth performance and apparent total tract nutrient digestibility of Holstein heifers limit-fed diets containing different amounts of wet brewer's grains (**WBG**). A 12-wk randomized complete block study was conducted using 30 yearling Holstein heifers [378 ± 27 d of age, and body weight (**BW**) of 357.8 ± 27.6 kg (mean ± SD)]. Treatments were 0%, 10% and 20% of WBG on a dry matter (**DM**) basis and diets were formulated to be limit-fed for dry matter intake (**DMI**) at 2.35% of BW and provided 15% crude protein (**CP**) and 2.27 Mcal ME / kg of DM. Dry matter intake was recorded daily while BW and skeletal measurements were measured every 2 wk. During wk 12, fecal samples were collected directly from the rectum over 4 consecutive days and composited by heifer to determine apparent total-tract nutrient digestibility using acid detergent insoluble ash as a marker. Data were analyzed using the MIXED procedure of SAS. Dry matter intakes, BW, and average daily gain were not different among treatments. Skeletal measurements and body condition scores (**BCS**) were not different among treatments except for the change in heart girth and initial BCS. Apparent total tract digestibilities of DM, organic matter, CP, crude fat and hemicellulose were greater or tended to be greater in heifers fed 0% and 20% WBG treatments than heifers fed 10 % WBG. Neutral detergent fiber and acid detergent fiber (**ADF**) digestibilities were similar among treatments. During the digestibility phase, DMI tended to be greater for the 10% WBG treatment. These results demonstrate that limit-feeding heifers with diets containing up to 20% WBG can replace soybean and corn-based concentrates in diets without adverse consequences to the heifer growth performance.

INTRODUCTION

Raising dairy heifers from birth to the first parturition is an expensive cost to producers because of relatively low feed efficiency and absence of income until the onset of lactation (Zanton and Heinrichs, 2009). Due to this, one of the priorities of dairy producers is to decrease the cost of production through strategies that optimize the growth of heifers without sacrificing productivity (Tozer and Heinrichs, 2001). Moreover, the cost of energy and protein feeds especially corn and soybean meal can be high; because of this, the substitution of corn and soybean meal for alternative feed sources is of great importance. One of the strategies to reduce production cost is to use less expensive feeds, such as wet brewer's grains (**WBG**). Wet brewer's grains are the by-products of the brewing industry, and they are a feasible alternative to corn and soybean products due to their nutritional value, low cost and availability in various regions of the country.

In terms of nutrition, the importance of WBG as a supplement may be attributed to its high protein content and low ruminal solubility (Homm et al., 2008). Wet brewer's grains are characterized by having approximately 28% CP, 47.1% NDF, 5.2% ether extract and high moisture content (NRC, 2001). Compared to energy content of ground corn (3.08 Mcal/kg of dry matter (**DM**), WBG contains about 2.60 Mcal/kg DM (Frasson et al., 2018). From an economic perspective, the current costs of the corn-based concentrates (energy mix) and soybean-based concentrates (protein mix) used at our research facility was estimated at $252 and $387/metric ton respectively (Poulin Grain, Inc., Newport, VT) while wet WBG cost was $190/metric ton on an as-dry basis. Nationwide, the current price for corn is $147.5 per metric ton, while soybean price is 333.5 per metric ton (Indexmundi, 2019).

Wet brewer's grains have been successfully used for feedlot heifers (Homm et al., 2008), and data indicated that feeding 15 to 45% WBG on a DM basis in feedlot diets supports performance and carcass characteristics similar to or greater than that in cattle fed a typical high moisture corn finishing diet. Research with lactating animals indicated that supplementing cows with WBG (15 and 30% of the diet) resulted in greater milk yield, fat and protein contents than cows supplemented with soybean meal (Murdock et al., 1981; Faccenda et al., 2017). Similar results were observed when corn silage was substituted with WBG at 9% (Belibasakis and Tsirogogianni, 1996). Greater milk yields and milk components are influenced by a better synchronization and presence of essential amino acids for milk synthesis such as lysine and methionine that are high in WBG (Faccenda et al., 2017). However, no research regarding the effect of feeding WBG to growing dairy heifers on their growth performance and nutrient utilization is available.

Another strategy to reduce the cost of raising dairy heifers is to use limit-feeding in which nutrient-dense diets are fed to meet but not to exceed nutrient requirements, reduce dry matter intake (**DMI**) and has the potential to increase nutrient digestibility while maintaining growth performance (Hoffman et al., 2007; Zanton and Heinrichs, 2009). Therefore, we hypothesize that replacing corn and soybean-based concentrates with WBG in dairy heifer's diet would provide similar growth performance. The objective of this experiment was to evaluate the effect feeding diets containing different amounts of WBG in limit-fed diets on the growth performance and total-tract nutrient digestibility of dairy heifers.

MATERIAL AND METHODS

Experimental Design

This experiment was reviewed and approved by the University of New Hampshire Animal Care and Use Committee (protocol # 170603). Thirty yearling Holstein heifers with a mean age of 378 ± 27 d and BW of 357.8 ± 27.6 kg (mean ± SD) were blocked by birth date and randomly assigned to 1 of 3 treatments (n =10) in a randomized complete block design. There were 3 heifers per block. Each heifer was on their respective treatment for 12 wk. The experiment was completed in a 10-mo period from March 2018 to January 2019 because heifers entered the study on or near their 12 months of age. Treatments were fed right away without using any adaptation period.

Treatment diets were formulated on a DM basis: (1) a control (0% WBG), (2) a diet containing 10% WBG and (3) a diet containing 20% WBG. Diets were formulated to be isonitrogenous (15% CP) and isocaloric (2.27 Mcal ME/kg of DM) using the NRC (2001) software to provide 0.9 kg/d of ADG. The fact that WBG has a high moisture content, a high protein content but also contributes as a fiber source in the diets, made it challenging to keep constant the ratio of grass silage:corn silage, and the ratio of the forage:concentrate across treatments. Moreover, rations were adjusted every time feed ingredients changed in nutrient content (DM, CP, NDF, ADF, crude fat, lignin, starch, Ca, P, Mg, K, S and Cl) during the entire course of the study.

Animal Care and Feeding

Heifers were group-housed in a naturally ventilated free-stall barn bedded with mattresses. One pen (15.9 × 4.8 m) having the capacity to host 16 heifers were used. Heifers were fed once daily at 0900 h using the Calan gate feeding system (American Calan Inc., Northwood, NH) in individual feed tubs to allow for feed intakes and refusals (if any). The feed was mixed and distributed using a motorized feeding vehicle (Data Ranger, American Calan Inc. Northwood,

NH). Before feeding, orts (if any) were collected from individual feed tubs and recorded. Samples of the total mixed ration (**TMR)** for each treatment were taken every day and stored at -20˚C for future nutrient analysis. Rations were limit fed to 2.35% of BW (DM basis) and adjusted every 2 wk based on BW measurements and DM analysis of the feed. To reach the targeted crude protein (**CP**) and energy content of diets, dietary DM was increased due to low-quality grass silage used in this experiment, but energy provision did not exceed the recommendations.

Heifers had ad libitum access to water through automatic refilling water troughs. Heifers were watched daily for any health problems and treated according to routine management of the research center, but no serious health problems were observed during the study.

Animal Measurements

Every 2 wk on Tuesday before feeding throughout the study, body growth measurements were recorded. Those included BW, body length, hip and withers height, heart and paunch girth, hip width and body conditions score (**BCS**) based on the scale described by Wildman et al., (1982) with 1 = emaciated and 5 = obese.

For BW, heifers were weighed on a platform scale (Cardinal, Northeast Scale Co. Inc., Hooksett, NH). Hip and wither heights were measured using a sliding height stick with a bubble level. Heart and paunch girth, as well as body length, were measured using a weight tape (Coburn Co, Inc., Whitewater, WI).

Feed and Fecal Samples Analysis

Frozen samples of the TMR and orts (orts were rare and only occurred during times of elevated environmental temperatures) were thawed and samples from 4 consecutive weeks were composited on an as-fed basis for each treatment as a monthly composite. Composites of samples

were then dried in duplicate for 48 h in a forced-air oven (Binder, Bohemia, NY) at 55°C and ground to a 1-mm screen Wiley mill (Model 3, Arthur H. Thomas, Philadelphia, PA). All samples were sent to a commercial laboratory (Rock River Laboratory Inc., Watertown, WI) for nutrient analysis. Samples were analyzed for neutral detergent fiber (**NDF**) (method 6 in an Ankom Fiber Analyzer A2000 with α-amylase and Sodium sulfite, Ankom Technology; solutions as in Van Soest et al., 1991), and acid detergent fiber (**ADF**) (method 5 in an Ankom Fiber Analyzer A2000, Ankom Technology, Fairpoint, NY; method 973.18, AOAC International, 1998). Hemicellulose was calculated as NDF% - ADF%. Nitrogen was analyzed via Dumas combustion (AOAC International 2002; method 968.06) on a Rapid N cube (Elementar Analysensystem, GmbH, Hanau Germany). Nitrogen was then multiplied by 6.25 to calculate the crude protein (**CP**). Starch concentration was analyzed using a modified method of glucose analysis (Bach Knudsen, 1997) completed on a YSI 2700 select Biochemistry Analyzer (YSI Biochemistry analyzer, YSI Inc., Yellow Spring, OH), and crude fat was analyzed with ether extraction technique (method 2003.05; AOAC International, 2006). Ash content was determined by incinerating 1 g of sample for 8 h at 450°C in a muffle furnace (AOAC International, 2002; method 942.05). Organic matter (**OM**) was calculated as OM = 100 - % ash. Mineral composition analysis included Ca, P, Mg, K, Na (AOAC International, 1998; method 985.01), and S (AOAC International, 1998; method 923.01).

Digestibility Measurements

Acid detergent insoluble ash (**AIA**) was used as an internal digesta marker to estimate 24 h fecal excretion, and total-tract nutrient digestibility was determined by calculations. Feeds, orts (if any) and feces were analyzed for ADF using the filter bag technique (method 5, Ankom Technology) followed by determination of acid-insoluble ash according to Van Keulen and Young (1977). The equation used to estimate apparent total tract nutrient digestibility was:

$$\text{Apparent total tract nutrient digestibility (\%)} = 100 - 100 \times \frac{\%\ \text{AIA in feed} \times \%\ \text{Nutrient in feces}}{\%\ \text{AIA in feces} \times \%\ \text{Nutrient in feed}}$$

Each of the 30 heifers underwent the digestibility phase at d 77 of study until d 84. Total mixed ration samples were collected on d 77 through d 81. Individual orts (if any) were collected on d 78 through d 82. Fecal samples were collected d 80 through d 84. Orts and TMR samples were then composited over the sampling days. Rectal fecal grabs samples (~ 200g / sample) were collected via gloved hand directly from the rectum for the last 4 d every 12 h to represent a 24-h period (d 80: 0900 and 2100 h; d 81: 1200 and 0000 h; d 82: 1500 and 0300 h; d 83: 1800 through 0600 h of d 84).

Fresh samples over the 4-d period were combined to obtain a single composite and were frozen at -20° C. Fecal samples were then thawed at room temperature and emptied into aluminum trays to be dried in a forced-air convection oven (Binder, Bohemia, NY) at 55° C for at least 96h until completely dried. The dried TMR, orts, and fecal samples were ground through a 1-mm screen using a Wiley mill (Model 3, Arthur H. Thomas). Ground samples were sent to Rock River Laboratory (Watertown, WI) for nutrient analysis. Fecal samples were analyzed for CP, ADF, NDF, crude fat, starch, ash, and AIA as previously described for feed samples. Neutral detergent fiber intake and NDF/BW, forage NDF intake, and forage NDF/BW were determined during the digestibility phase (wk 12) to determine if there were differences due to varying amounts of NDF in the diets.

Statistical Analysis

Initial BW and skeletal measurements served as covariates for their respective variables of interest. Growth characteristics (BW, body length, hip and withers height, heart and paunch girth, hip-width and BCS) were analyzed as randomized complete block design with week as repeated

measure and heifer (block) as subject using the Mixed procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). The model included treatment, week, and treatment × week interaction as follows:

$Y_{ijkl} = \mu + B_i + H_j + T_k + W_l + TW_{kl} + X_{ijk} + E_{ijkl}$,

where Y_{ijkl} = the dependent variable; μ = the overall mean; B_i = the random effect of block i (i = 1, . . ., 10); H_j = the random effect of heifer (j = 1, ...,10) T_k = the fixed effect of the k_{th} treatment (k = 0; 10 and 20% WBG); W_l = the fixed effect of the l_{th} week of the study (l = 2, 4, 6, 8, 10, 12); TW_{kl} = the fixed effect of interaction between the k_{th} inclusion rate of WBG and the l_{th} week; X_{ijk} = the covariate measurement; and the E_{ijkl} = the residual error. Compound symmetry, unstructured, first-order autoregressive, variance components and Toeplitz covariance structures were determined and the structure with the lowest Bayesian information criterion was chosen. Degrees of freedom were calculated using the Kenward-Roger approximation option of the Mixed procedure. Single degrees of freedom contrasts for linear and quadratic effects were determined. If the covariate analysis resulted in a probability > 0.25, the covariate was removed from the model. Dry matter intake, ADG, and gain-to-feed ratio were analyzed similarly but without covariate adjustment.

Initial and final BW, hip and withers heights, heart and paunch girth, hip width, and BCS were analyzed as randomized complete block design using the Mixed procedure of SAS 9.4 (SAS Institute Inc.) according to the following model:

$Y_{ijk} = \mu + B_i + T_j + X_{ij} + E_{ijk}$,

Where Y_{ijk} = the dependent variable; μ = the overall mean; Bi = the random effect of block I (i = 1, . . ., 10); T_j = the fixed effect of the j_{th} inclusion rate of WBG (0, 10 and 20%); X_{ij} = the covariate measurement; and E_{ijk} = the residual error.

Changes over time for the growth parameters were calculated for the 2-wk intervals and averages analyzed using repeated measures in the Mixed procedure of SAS. The gain-to-feed ratio was calculated as the ratio of ADG to DMI for each treatment.

Apparent total-tract digestibility data was analyzed using the Mixed procedure of SAS 9.4 (SAS Institute, Inc.,) according to the following model:

$Y_{ijk} = \mu + B_i + T_j + E_{ijk}$,

where Y_{ijk} = the dependent variable; μ = the overall mean; B_i = the random effect of block I (i = 1, . . ., 10); T_j = the fixed effect of the j_{th} inclusion rate of WBG (0, 10 and 20% WBG); and E_{ijk} = the residual error. Regression coefficients and quadratic maxima/minima of dietary wet brewer's grains for variables with significant quadratic effects were calculated using the equation by Brito and Broderick, 2006): [Linear coefficient: (2×Quadratic coefficient)].

For all variables, the least-square means for each treatment were reported. Significant treatment and interaction effects were declared at $P \leq 0.05$ and a tendency was declared at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

The goal of this experiment was to evaluate the growth performance and total-tract nutrient digestibility in yearling Holstein heifers limit-fed with diets containing different amounts of WBG. Much research has been conducted looking at the effects of supplementing WBG in lactating cows' diets to evaluate milk production and milk composition (Murdock et al., 1981; Berbasakis and Tsirogogianni, 1996), and showed greater milk yield and greater fat and protein content in cows supplemented with WBG. Moreover, Homm et al. (2008) evaluated the corn silage replacement value with WBG and found improved performance and carcass characteristics similar to or greater

than that in cattle fed a typical high moisture corn finishing diet but we could not find any research evaluations on feeding WBG to dairy heifers.

Feed Nutrients and Diet Composition

The average nutrient composition of major feed ingredients is presented in Table 6. Because of different entrance dates of different groups (blocks) of heifers, the experiment that included all 30 heifers was completed in a 10 mo period, and every block of 3 heifers stayed in the experiment for 12 weeks. Therefore, the analysis of major feed ingredients is shown with standard deviations, indicating nutrient variation throughout the experiment. The ingredient of experimental diets is listed in Table 7 and treatments were formulated to be isonitrogenous and isocaloric.

Treatments were built based on results from previous studies conducted on feedlot heifers, finishing steers and lactating dairy cows which suggested that feedlot heifers fed diets containing 15 to 30% WBG had improved carcass characteristics compared to heifers fed high concentrate diets (Homm et al., 2008). Parmenter et al. (2018) suggested that low-inclusion of WBG (7% WBG) in diets can support the growth performance and carcass characteristics of finishing cattle, while lactating cows fed diets containing 15 to 30% WBG had improved milk yield compared to dairy cows fed normal lactating diets (Murdock et al., 1981; Faccenda et al., 2017). In our current study, we limited the amount of WBG to be included in our diets at a maximum of 20%, and at the minimum of 10% WBG to avoid any negative effect on DM intake when greater amounts of WBG are included in the diets.

In diets of the current study, the combination of grass silage and corn silage contributed to the forage content of 80.7%; 78.8%, and 74.1% for diets 0%, 10% and 20% WBG respectively. The 20% WBG diet had a lower forage content due to increased amounts of WBG. The

contribution of WBG as a fiber source is not negligible (Firkins et al., 2002), that's why reducing the amount of forage concentration has been considered with increasing levels of WBG in diets. The fact that WBG has high moisture and high protein content, as well as a highly digestible fiber content makes it hard to make a balanced diet especially with high fiber diets in which large amounts of WBG need to be consumed to provide adequate nutrients (Hersom, 2006). Hence, our diets exhibited changes in forage to concentrate ratio to provide similar nutrient content across treatments. The ratio of grass silage:corn silage was 1.58; 1.87 and 1.82 for 0, 10 and 20% WBG diet respectively, which made NDF intake less for heifers fed 0% WBG, and forage NDF intake less for heifers fed 20% WBG respectively.

Energy mix and protein mix inclusions were decreased or removed from diets as the amount of WBG increased from 0 to 20% to keep protein and energy levels balanced (Table 7). All diets were formulated to be similar in all nutrients except the DM which decreased as the inclusion of WBG increased, and this was due to the high moisture content of WBG. On average, diets provided 2.29 ME Mcal/kg DM which was closer to the targeted energy intake of 2.27 ME Mcal/kg DM. Based on feed ingredient prices, the cost of treatment diets was on average $0.26; $0.22 and $0.20/kg of DM for 0%, 10% and 20% WGB diets respectively.

Table 8 contains the nutrient composition of diets based on laboratory analysis. Organic matter was similar in all diets while CP content in the control diet was slightly lower (14.6% CP) than the target of 15% CP. Crude protein content in 10% WBG diet was on target while CP content was higher (15.9% CP) in the 20% WBG diet. Wet brewer's grains varied in the DM and nutrient content (CP, NDF, ADF, crude fat, lignin, starch, Ca, P, Mg, K, S and Cl) throughout the study as it was purchased in different batches. The variation in the nutrient content of WBG suggests a constant nutrient analysis for better inclusion in diets because grain varieties and

brewing processes have an important influence on nutrient composition of WBG (Robertson et al., 2010; Muthusamy, 2014). Moreover, small batches of WBG were used in this study to avoid much nutrient loss observed when large batches of WBG are delivered and get spoiled by yeast and mold if no preservative is applied such as commercial preservatives (Marston et al., 2009), salt (Hatungimana and Erickson, 2019), or ensiling inoculants (Lilly et al., 1980; Schneider et al., 1995), or ensiling with dried feeds (Nishino et al., 2003). The high moisture content of WBG is the main limitation for long period storage and use in livestock feeding (Wang et al., 2014).

Average NDF and ADF were consistent in all diets while starch and NFC were greater in the control diet (0% WBG) because of energy mix inclusion. Crude fat was slightly greater in the 20% WBG diet due to the amount of WBG used and its high-fat content (7.2% fat). Variation in the nutrient composition of the rations over time was observed; however, rations provided adequate nutrients and were comparable to typical diets fed to growing heifers. Phosphorous concentrations were elevated in diets due to the feeding rate of the mineral mix (limit-fed heifer diet) and a greater concentration of P in the WBG.

Heifer Growth Performance

Results for BW, DMI, ADG and feed efficiency (Gain:Feed) are presented in Table 9. Treatment effect and treatment × week interactions were not found for those parameters. The average initial BW of heifers was not different ($P = 0.64$) which means that heifers entered the study with similar BW. The average BW was not different among treatments and no treatment × week interaction was found. The final BW was also not different among treatments meaning that heifers consumed similar amounts of nutrients leading to similar growth rates. Overall, heifers fed the control (0%), 10 and 20% WBG increased their BW by 22.5, 24.7, and 24.4% respectively.

Dry matter intakes were similar among treatments ($P = 0.22$) and treatment x week interaction was not observed. However, DMI increased over time because heifers were limit-fed based on a percentage of BW. Similar DMI were reported by Hoffman and Armentano (1988), when feeding up to 25% dried brewer's grains to lactating cows. However, Davis et al. (1983) observed a decrease in DMI when 30 to 40% of WBG were fed to lactating dairy cows. In contrast to our results, Homm et al. (2008) observed greater DMI and ADG in beef heifers fed 15 and 30% WBG than beef heifers fed the control and 45% WBG.

Average daily gain and feed efficiency were slightly greater than the targeted gain of 0.90 kg/d. Moreover, ADG and feed efficiency were similar among treatments and decreased over time as nutrient requirements for maintenance increased with BW (Anderson et al., 2015). Despite that dietary energy intake was closer to the target, heifers had greater ADG than recommended and we think that NRC (2001) model used to formulate diets may have overestimated the energy requirements of growing heifers or underestimated the energy provided by feed ingredients. Similar results were reported by Anderson et al. (2015a, b) and Manthey et al. (2016) who limit-fed diets containing distiller's grains with different forage to concentrate ratios to growing dairy heifers. Our diets provided greater energy than expected which would have probably caused greater ADG than was expected. Regardless of feeding strategy, Zanton and Heinrichs (2005) recommend dairy heifers to be fed energy to allow the ADG of 0.8 to 0.9 kg/d.

Skeletal measurements and BCS are presented in Table 10. Similar to BW findings, heifers' skeletal measurements were not different among treatments and increased throughout the study. No difference was observed in change per day for the skeletal measurements except for hip width change that had a treatment × week effect ($P = 0.03$). This effect was not expected as

there were no treatment or treatment × wk effect on overall mean hip width, thus suggesting that heifers were consuming adequate amounts of nutrients to promote growth throughout the study. Initial withers height and BCS were different (P = 0.02 and P = 0.01 respectively), heifers with the greatest wither's height being on the 10% WBG treatment. Initial paunch girth tended to be different (P = 0.10) and heifers fed the 20% WBG had the least paunch girth. However, there were no differences in final skeletal measurements indicating similar growth performance among treatments. Body weight gain per cm of hip height gain (kg/cm daily) was calculated to check if heifers were growing or fattening and those ratios were similar among treatments. No treatment or treatment × wk effect was observed on BW gain per cm of hip height gain.

On average, heifers grew approximately 9.7 cm in body length, 6.8 cm withers height, and 5.8 cm in hip height throughout the experiment. Body condition scores slightly increased for all heifers but were similar across treatments. The similarity of frame growth agrees with findings from other research on limit-feeding when heifers have similar energy intakes (Zanton and Heinrichs, 2007).

Dry matter intakes and Total –Tract Nutrient Digestibility as measured during wk 12

The dry matter intake, NDFI and forage NDFI as well as DMI as a percentage of BW, NDFI as a percentage of BW and forage NDF as % BW, and the total-tract nutrient digestibility of diets measured during wk 12 are presented in Table 11.

Dry matter intake tended to be different among treatments (P = 0.08) and had a quadratic response (P = 0.04, and a quadratic maximum at 8.33% WBG) with less DMI in heifers fed the 20% WBG diet. Regardless of small particle size, Firkins et al. (2002) found WBG could also be an effective replacement of the forage NDF, which could have contributed to more gut fill in

heifers fed 20%WBG. From these results, feeding 20%WBG in the diet of growing heifers would likely decrease DMI. Moreover, Schingoethe et al. (1988) found that the moisture content of WBG can affect the level of intake in cattle, particularly when it is fed in combination with silages. The lower DMI observed in heifers fed 20% WBG may be a response to gut fill and distension caused by the structural volume of WBG water held within the cell wall (Balch and Campling, 1962). However, DMI as a percentage of BW was similar among treatments.

Total NDFI was greater for heifers fed 10% and 20% WBG ($P < 0.01$) and had a quadratic response ($P < 0.01$), with the quadratic minimum of 11.25% WBG (Table 12). Forage NDF intake as provided by grass silage and corn silage was different among treatments ($P < 0.01$) with a linear and quadratic effect ($P < 0.01$, $P = 0.01$ respectively) and was less for heifers fed 20% WBG. The quadratic minimum for forage NDFI was 7.5% WBG (Table 12). This was expected due to the increased amount of WBG in the diet. According to Hersom (2006), the effective fiber of WBG is useful in dairy rations as it is utilized to replace some portion of the forage in the rations. Because most of the starch has been fermented away, WBG can be considered as a moderate source of fiber.

Neutral detergent fiber intake as a percentage of BW was different among treatments ($P = 0.01$) and was less in heifers fed the control diet (0%WBG). According to Hoffman and Kester (2013), dairy heifers consume a near-constant 1.0% of BW as NDF, which agrees with our results (Table 6). Neutral detergent fiber intakes conform to gut fill theories of intake regulation according to Mertens (1994) who suggests that gut fill regulation of NDFI occurs in lactating dairy cows at 1.2% of BW when fed diets containing > 30% NDF. Mertens et al. (1994), fed diets containing 36.1 to 49% NDF (greater than NDF in diets typically fed to lactating cows) to heifers, and found that NDF gut fill regulation occurred near 1% of BW.

The digestibility of DM and OM was different among treatments ($P = 0.04$ and $P = 0.04$ respectively) and had a quadratic response ($P = 0.01$ and $P = 0.01$, with a quadratic minimum at 9.26 and 10.95% WBG respectively). Greater DM and OM digestibility was observed in heifers fed the 10% WBG diet. Crude protein digestibility tended to be different among treatments ($P = 0.06$) and had a quadratic effect ($P = 0.1$) and was greater in heifers fed the 0% WBG and the 20% WBG diet compared to heifers fed 10% WBG diet ($P < 0.03$). The digestibility of NDF and ADF was similar among treatment.

According to Colucci et al. (1982), the rate at which digesta move through the gastro-intestinal tract, the rate of fermentation of the feed, and the amount of DM consumed are the major factors that determine how much of the nutrient will be digested, absorbed and utilized in the animal. Alteration of one of those factors generally changes the other two. The lower DM digestibility observed in heifers limit-fed diets with 10% WBG would likely be attributed to greater forage NDF content in the diet (Pino et al., 2018). Including WBG in heifers' diets increased fiber content, because WBG contains considerable NDF content despite its small particle size (Firkins et al., 2002). Greater NDF content in the diet causes a slower passage rate and increased retention time in the rumen, which may allow microbial growth and more nutrient absorption (Pino et al., 2018). This may explain the similar growth performance of heifers fed 10% WBG despite less nutrient digestibility observed in this group.

Results of DM digestibility of diets from this study were lower (53.6, 44.8 and 51.2% for the 0%, 10%, and 20% WBG respectively) compared to 77% DM digestibility when WBG was included up to 15% in diets composed of corn silage, ryegrass silage, and concentrated feed which were provided to Holstein cows (Geron et al., 2010). Greater CP digestibility in the 20%

WBG diet may have been influenced by greater RUP digestibility of WBG. According to Clark et al. (1987), approximately 50% of the protein found in WBG is RUP.

Digestibilities of NDF, ADF, and starch were similar among treatments. Fat digestibility tended to be different among treatment ($P = 0.06$) and increased linearly ($P = 0.05$) with increasing levels of WBG in diets. Fat digestibility was greater in heifers fed the 20% WBG. Fat from WBG is bound to feed particles and slowly introduced to the rumen and has fewer negative effects compared with other dietary fat sources (Westendorf et al., 2002). Hemicellulose digestibility was different among treatments ($P < 0.01$) and had a quadratic response ($P = 0.007$, with a quadratic minimum at 10.91% WBG) and was greater in heifers fed the 0% WBG and the 20% WBG diets compared to heifers fed the 10% WBG diet.

Overall, the digestibility of DM, OM, CP, fat, and hemicellulose was less in heifers fed 10% WBG probably because of greater grass silage:corn silage (1.58, 1.87 and 1.82 for 0, 10 and 20% WBG diet respectively) leading to greater NDF and forage NDF intake.

CONCLUSION

In agreement with our hypothesis, dairy heifers performed equally when limit-fed diets containing different levels of WBG. However, we suggest not to feed more than 20% WBG in diets of dairy heifers as we observed a decreasing tendency in the performance of heifers fed greater amount of WBG. Body weight and frame growth were similar among treatments but ADG was slightly greater than the target. Nutrient digestibilities were mostly greater for heifers fed the 0% and 20% WBG diet suggesting that grass silage and WBG used could have influenced the overall digestibility as they contributed to greater NDF content in the 10% WBG. This research indicates that dairy producers can use WBG at a rate of up to 20% DM to replace

soybean and corn-based supplements without compromising heifer growth performance. Based on feed ingredient prices, the cost of treatment diets was on average $0.26; $0.22 and $0.20/kg of DM for 0%, 10% and 20% WGB diet respectively. Considering the ADG of heifers fed different amounts of WBG in diets, the feed cost per kg of ADG was $2.21, $1.90 and $1.79 for heifers fed 0, 10 and 20% WBG diet respectively. Hence, feeding diets containing 20% WBG to dairy heifers would be more economical than feeding diets supplemented with conventional concentrates.

Table 6**:** Average nutrient composition of major ingredients used in the experiment

Item, % DM	Grass silage	Corn silage	WBG[1]	Energy Mix[2]	Protein Mix[3]
DM	30.1 ± 3.5	31.9 ± 2.9	24.9 ± 2	87.9	88.5
CP	13.6 ± 2.7	7.4 ± 0.6	31.8 ± 0.3	7.8	53.2
NDF	58.9 ± 5.9	41.2 ± 3.4	46.3 ± 1.2	21.8	14.8
ADF	38.6 ± 3.7	24.8 ± 2	21 ± 0.03	12.5	9.3
Lignin	5.7 ± 0.6	3.1 ± 0.3	-	-	-
Starch	1.1 ± 0.3	32.1 ± 3	2.1 ± 0.04	-	6.4
NEm (Mcal/kg DM)	1.4 ± 0.1	1.68 ± 0.06	1.75 ± 0.1	1.2	-
NEg Mcal/kg DM)	0.76 ± 0.13	1.06 ± 0.05	1.12 ±0.1	1.8	-
Fat	4.2 ± 0.5	3.0 ± 0.2	7.2 ± 0.05	3.5	2.6
Ash	8.4 ± 0.9	3.4 ± 0.4	4.7 ± 0.03	3.0	-
Ca	0.68 ± 0.06	0.19 ± 0.2	0.21± 0	0.40	0.55
P	0.43 ± 0.18	0.22 ± 0.01	0.63± 0	0.22	0.83
K	2.6 ± 0.12	0.9 ± 0.4	0.07 ± 0	0.58	2.12
Mg	0.1 ± 0.02	0.1 ± 0.02	0.19 ± 0	0.16	0.40
S	0.2 ± 0.02	0.1 ± 0.0	0.32 ± 0	0.17	0.48

[1]WBG = Wet Brewer's Grains, a by-product of the beer brewing industry

[2]Contained 5% pellet mill molasses; 45.79% fine corn meal; 15.2% steam flaked corn and 33.99% whole beet pulp. Delivered in large batches and no variations in nutrient content

[3]Contained 7.28% distillers; 69.14% soybean meal; 21.83% canola and 1.75% urea

Table 7: Ingredient composition (% DM) of experimental diets containing 0, 10 or 20% wet brewer's grains limit- fed to yearling heifers

	Treatments[1]		
	0% WBG	10% WBG	20% WBG
Ingredient, % of DM			
Energy mix[2]	2.17	0.00	0.00
WBG[3]	0.00	10.00	20.00
Protein mix[4]	11.82	5.51	0.00
Grass silage	49.44	51.36	47.84
Corn silage	31.26	27.43	26.21
Mineral mix[5]	5.32	5.69	5.95

[1]Formulated according to the NRC (2001)

[2]Contained 5% pellet mill molasses; 45.79% fine corn meal; 15.2% steam flaked corn and 33.99 % whole beet pulp

[3]WBG = Wet Brewer's Grains, a by-product of the beer brewing industry

[4]Contained 7.28% distillers; 69.14% soybean meal; 21.83% canola and 1.75 % urea

[5]Contained 19.05 % Ca; 6.01 % P; 3.51 % Mg; 20.00 % Salt; 7.80 % Na; 0. 26 % Fe; 0.26% Zn; 0.26 % Mn; 12.30% Cl; 602 mg/kg Cu; 15.0 mg / kg Co; 25.09 mg/kg Se; and 15.00mg/kg I; 267,800 IU/kg vitamin A; 111,071 IU/kg vitamin D; and 2,207 IU/kg vitamin E

Table 8: Laboratory nutrient composition of experimental diets

Item, % DM	Treatments		
	0% WBG	10% WBG	20% WBG
DM	36.7 ± 2.2	34.3 ± 2.1	31.2 ± 1.1
OM	88.1 ± 0.2	88.8 ± 0.1	88.9 ± 0.1
CP	14.6 ± 0.2	15.2 ± 0.2	15.9 ± 0.3
aNDF[1]	48.3 ± 0.3	51.2 ± 0.4	49.2 ± 0.2
ADF	29.7 ± 0.8	30.9 ± 0.6	29.6 ± 0.8
Fat [2],	3.0 ± 0.0	3.2 ± 0.3	3.6 ± 0.0
Starch	13.6 ± 0.0	12.1 ± 0.5	10.7 ± 0.3
[3]NFC	30.5 ± 0.6	27.0 ± 0.4	26.9 ± 0.1
NEG, Mcal/kg DM	0.95 ± 0.3	0.93 ± 0.02	0.95 ± 0.03
Ash	11.8 ± 0.1	11.2 ± 0.1	11.1 ± 0.1
Ca	1.09 ± 0.00	1.01 ± 0.05	1.09 ± 0.02
P	0.78 ± 0.01	0.74 ± 0.00	0.80 ± 0.00
K	1.86 ± 0.05	1.69 ± 0.03	1.46 ± 0.01
Mg	0.37 ± 0.00	0.35 ± 0.00	0.35 ± 0.00
Cl	1.26 ± 0.00	1.13 ± 0.00	1.19 ± 0.00
S	0.32 ± 0.00	0.30 ± 0.00	0.33 ± 0.00

[1]α-amylase NDF

[2] Ether extract

[3] % NFC = 100 – (CP% + (NDF% – neutral detergent insoluble CP%) + fat% + ash%)

Table 9**:** Dry matter intakes, BW and gain-to-feed for dairy heifers limit-fed diets containing 0, 10 or 20% wet brewer's grains.

	Treatment (WBG %)				***P*-values**				
Item	0%	10%	20%	SEM	Trt	Wk	Trt ×Wk	L	Q
Age (d ± SD)[1]	362.2 ± 22.8	372.0 ± 29.2	365.1 ± 24.1						
BW, kg									
Mean	404.4	411.5	409.3	3.77	0.41	< 0.01	0.19	0.38	0.32
Initial	359.4	357.4	352.2	5.4	0.64				
Final	440.2	445.7	438.2	5.2	0.58			0.80	0.31
DMI, kg	8.8	9.0	8.6	0.14	0.22	< 0.01	0.69	0.47	0.12
ADG	1.03	1.04	0.96	0.06	0.59	< 0.01	0.22	0.40	0.53
Gain:Feed	0.12	0.12	0.11	0.006	0.79	< 0.01	0.14	0.56	0.81

[1]Initial age at the start of the study

Table 10: Skeletal measurements for dairy heifers limit-fed diets containing 0, 10 or 20% wet brewer's grains

Item	Treatments (WBG %)			SEM	*P*-value				
	0%	10%	20%		Trt	Wk	Trt × Wk	L[1]	Q[2]
Withers height									
Mean, cm	137.4	137.1	136.8	0.36	0.40	< 0.01	0.37	0.18	0.92
Initial	132.3	135.1	131.4	1.3	0.02			0.51	0.01
Final	140.0	139.6	139.6	0.49	0.72			0.48	0.76
Change[3], cm/d	0.09	0.08	0.08	0.01	0.16	0.15	0.87	0.25	0.42
Hip height									
Mean, cm	140.2	140.6	140.4	0.34	0.75	< 0.01	0.54	0.66	0.55
Initial	137.3	137.4	136.4	0.64	0.47			0.32	0.47
Final	142.6	143.2	142.6	0.49	0.56			0.98	0.29
Change[3], cm/d	0.06	0.07	0.06	0.007	0.62	0.97	0.58	0.96	0.33
Body length									
Mean, cm	127.2	126.7	128.1	0.66	0.34	< 0.01	0.68	0.34	0.26
Initial	122.9	123.0	120.8	0.98	0.20			0.13	0.33
Final	133.1	131.3	131.3	1.17	0.44			0.28	0.53
Change[3], cm/d	0.14	0.10	0.14	0.03	0.5	0.1	0.77	0.94	0.25
Heart girth									
Mean, cm	174.7	173.9	174.2	0.82	0.78	< 0.01	0.41	0.66	0.59
Initial	167.2	168.8	165.5	1.40	0.27			0.40	0.17
Final	181.1	179.1	179.3	1.23	0.45			0.30	0.50
Change[3], cm/d	0.22	0.14	0.13	0.02	0.01	0.47	0.46	0.01	0.19
Paunch girth									
Mean, cm	205.4	205.0	207.5	1.30	0.36	< 0.01	0.85	0.26	0.34
Initial	197.8	198.0	192.8	0.91	< 0.10			0.07	0.26
Final	211.5	209.6	212.3	1.9	0.61			0.60	0.26
Change[3], cm/d	0.18	0.22	0.18	0.04	0.73	0.17	0.25	0.85	0.44
Hip width									
Mean, cm	47.5	47.8	47.7	0.17	0.31	< 0.01	0.14	0.44	0.18
Initial	46.5	46.3	46.1	0.26	0.42			0.19	0.95
Final	48.4	48.9	48.3	0.26	0.26			0.78	0.11
Change[3], cm/d	0.02	0.03	0.02	0.003	0.23	0.62	0.03	-	-
BCS[4]									
Mean, cm	3.6	3.6	3.6	0.04	0.9	< 0.01	0.61	0.70	0.88
Initial	3.5	3.4	3.4	0.04	< 0.01			0.16	0.01
Final	3.7	3.8	3.6	0.07	0.32			0.52	0.23

[1]Linear effect

[2]Quadratic effect

[3]Calculated based on change per 2-wk interval.

[4]BCS = Body condition score (1-5)

Table 11**:** Dry matter intake and apparent total tract nutrient digestibility of nutrients for heifers limit-fed diet containing 0, 10 or 20% WBG during week 12.

	Treatment (WBG[1])				*P*- value		
Item	0%	10%	20%	SE	Treatment	L[2]	Q[3]
DMI[4], kg/d	9.57	10.0	9.23	0.2	0.08	0.28	0.04
NDFI[5], kg/d	4.32	4.83	4.57	0.01	0.01	0.1	0.01
Forage NDFI, kg/d	4.15	4.27	3.72	0.09	< 0.01	0.001	0.01
DMI, % BW	2.12	2.20	2.15	0.05	0.21	0.76	0.24
NDFI, % BW	0.98	1.07	1.05	0.02	0.01	0.03	0.03
Forage NDFI, % BW	0.94	0.95	0.85	0.01	< 0.01	< 0.01	0.02
DM[6], %	53.6	44.8	51.2	2.3	0.04	0.46	0.01
OM[7], %	57.9	49.7	55.2	2.1	0.04	0.36	0.01
CP[8], %	45.3	39.5	50.1	2.9	0.06	0.25	0.03
NDF[9], %	47.0	40.7	45.2	2.5	0.22	0.62	0.09
ADF[10], %	37.4	30.9	35.7	3.1	0.29	0.70	0.13
Fat[11], %	66.8	65.6	72.3	2.2	0.06	0.05	0.15
Starch[12], %	98.5	98.2	98.7	0.2	0.28	0.47	0.15
Hemicellulose[13], %	58.2	50.8	55.9	1.7	0.01	0.36	0.007

[1]WBG = Wet brewer's grains, a by-product of the beer brewing industry.

[2]Linear effect

[3]Quadratic effect

[4]DMI = Dry matter intake (kg/d) during the digestibility phase

[5]NDFI = Neutral detergent fiber intake

[6]DM = Dry matter digestibility

[7]OM = organic matter digestibility

[8]CP = crude protein digestibility

[9]NDF = Neutral detergent fiber digestibility

[10]ADF = Acid detergent fiber digestibility

[11]Fat digestibility

[12]Starch digestibility

[13]Hemicellulose digestibility

Table 12. Regression coefficients and quadratic maxima and minima of dietary wet brewer's grains for variables with significant quadratic effects on wk12

Item	Intercept	SE	Linear coefficient	SE	Quadratic coefficient	SE	% Quadratic Minima/maxima, % WBG[1]
DMI[2]	9.54	0.37	0.10	0.09	-0.006	0.004	8.33
NDFI[3]	4.33	0.18	0.09	0.04	-0.004	0.002	11.25
Forage NDFI	4.16	0.16	0.05	0.04	-0.003	0.002	7.5
DM[4]	53.66	2.92	-1.63	0.74	0.075	0.04	9.26
OM[5]	57.96	2.61	-1.49	0.66	0.068	0.03	10.95
CP[6]	45.27	3.17	-1.39	0.81	0.082	0.04	8.48
Hemicellulose[7]	58.25	2.28	-1.37	0.58	0.06	0.03	10.91

[1](-Linear coefficient) ÷ (2 ×Quadratic coefficient)

[2]DMI = Dry matter intake (kg/d) during the digestibility phase

[3]NDFI = Neutral detergent fiber intake

[4]DM = Dry matter digestibility

[5]OM = Organic matter digestibility

[6]CP = Crude protein digestibility

[7]Hemicellulose digestibility

CHAPTER IV

Effect of storage of wet brewer's grains with incremental levels of salt on apparent total-tract nutrient digestibility and purine derivative excretion in dairy heifers

Abstract

The objective of this study was to evaluate the apparent total-tract nutrient digestibility and purine derivative excretion in heifers fed diets containing wet brewer's grains (**WBG**) treated with salt. A 12-wk replicated 4×4 Latin square study was conducted using 8 Holstein heifers of 224.5 ± 19.4 d of age, and body weight (**BW**) of 219.2 ± 28.1 kg (mean ± SD). Fresh WBG were treated with 0%, 0.8%, 1.6% and 2.4% and were stored for 4 days before being included in the diet at 20% on a dry matter (**DM**) basis. The diet was composed of 9% grass silage, 47% corn silage, 19% corn meal, 2% soybean meal (SBM) and 3% mineral mix. Additional salt was added to the diet at the time of mixing to achieve a similar salt concentration. Diets (DM basis) were formulated to be limit-fed at 2.15% of BW to provide 14% crude protein (**CP**) and 2.27 Mcal ME/kg of DM. Heifers were adapted to diets for 14 d followed by a 7-d collection period. Dry matter intake (**DMI**) was recorded daily during the collection week while BW was recorded weekly. Urine and fecal samples were collected during the last 4 d of the collection period and composited by heifer to determine apparent total-tract nutrient digestibility using acid detergent insoluble ash as a marker. Dry matter intake was greater in heifers fed 1.6% salt diet and was 5.13 kg DM /d vs 5.09 kg DM/d for the control diet. Body weight, ADG and feed efficiency increased linearly with increasing levels of salt in WBG. Digestibility of DM, OM and CP linearly decreased with increasing levels of salt in WBG. Compared to the control, apparent digestibility decreased by 22.2, 24.2 and 25.5% for DM, OM and CP respectively for heifers fed 2.4% salt. Urinary volume, allantoin, and uric acid excretion and total purine derivative excretion were not affected by treating WBG with salt. Mold counts and yeasts were not different among treatments. The losses in WBG linearly decreased as

salt increased. The results of this study demonstrate that limit feeding heifers with diets containing WBG treated with salt decreased apparent total tract digestibility of DM, OM and CP but enhanced heifer growth performance. Moreover, salt treatment may decrease WBG losses during storage despite that molds and yeasts were not affected by salt inclusion in WBG.

INTRODUCTION

The high moisture content of WBG is associated with high transportation costs and a short shelf-life of storage. Different strategies for WBG conservation have been examined such as drying (Pereira et al., 1998), ensiling with dried feeds (Kung, 2005), the addition of bacterial inoculants (Marston et al., 2009), and storage with salt (Hatungimana and Erickson, 2019). However, these conservation techniques of WBG have not been evaluated for their effect on nutrient digestibility in vivo. Hatungimana and Erickson (2019) reported that storing WBG with salt resulted in greater in vitro DM digestibility and in situ DM and protein degradability.

Salt has been proven to be associated with liquid dilution rate and increased efficiency of microbial protein synthesis, increased fiber digestibility and milk fat synthesis, and increased OM utilization in cattle fed high concentrate diets (Harrison et al., 1975; Rogers et al., 1982; Schneider et al., 1988). Based on results of a previous study (Hatungimana and Erickson, 2019), we hypothesize that treating and storing WBG with salt would enhance dietary nutrient digestibility and improve microbial protein synthesis.

The current study aims at evaluating the apparent total-tract nutrient digestibility and urinary purine derivatives (**PD**) as an indicator of microbial crude protein synthesis in dairy heifers limit-fed diets containing WBG treated with salt.

MATERIAL AND METHODS

Experimental design

This experiment was reviewed and approved by the University of New Hampshire Animal Care and Use Committee. Eight Holstein heifers with a mean age of 224.5 ± 19.4 d and BW of 219.2 ± 28.1 kg (mean ± SD) were used in a replicated 4 × 4 Latin square and randomly assigned to 4 treatments. Each period lasted for 21 d, of which 14 d of adaptation and 7 d of sample collection.

Fresh WBG was collected weekly from a local dairy farm and transported (18.3 km) to the Fairchild Dairy Research Center at the University of New Hampshire (Durham). One sample of fresh WBG was collected from the weekly batch and sent to ANALAB (Fulton, IL) for yeast and mold count analysis (AOAC International, 1999, method 997.02, as described by Hatungimana and Erickson, 2019). Another fresh sample of WBG was dried in an air-forced oven for 48 h and sent to the Rock River laboratory (Watertown, WI) for nutrient analysis.

After arrival at the research center, the WBG was treated with 4 amounts of salt (0, 0.8, 1.6, or 2.4%) on a DM basis considering the 20% DM inclusion in the diet. The salt amounts and the feeding rate of WBG were based on previous studies by Hatungimana and Erickson (2019, unpublished data). Treatments were formulated considering the maximum tolerable salt in diets of dairy cattle which is around 40g NaCl/kg DM or 1.40 g NaCl/kg BW (NRC, 2001). Our diets provided approximately 29 g NaCl/kg DM.

Treated WBG was mixed using a motorized feeding vehicle (Data Ranger, American Calan Inc. Northwood, NH) for 5 min and stored for 4 d in plastic tubs inside a commodity shed. Initially, WBG was intended to be stored for a week which is a typical feedout length of WBG in the North

East region of the United States. However, the storage time was reduced to 4 d due to a rapid spoilage of WBG observed during high environmental temperatures.

Plastic tubs containing treatments (~ 80 kg of WBG each) were weighed on a platform scale (Cardinal, Northeast Scale Co. Inc., Hooksett, NH) before storage and after the collection period to measure the amount of WBG lost (fresh basis) due to evaporation and growth of mold and yeast. After 4 d of storage, treatments were included in the TMR at the rate of 20% DM of the diet by the time of feeding. During the collection period, plastic tubs containing WBG were weighed at the beginning of storage and at the end of the collection period to determine the weight loss of WBG (fresh basis) due to spoilage by mold or water evaporation. Amounts of WBG removed from each feeding was recorded. The WBG was stored 4 d before being included in the diets to allow the interaction of salt and WBG and minimize losses in non-treated WBG (0% salt). During feeding, additional salt was added to diets at the time of mixing to achieve a similar salt concentration (Table 2).

Animal care and feeding

Heifers were group-housed in a naturally ventilated free-stall barn bedded with mattresses in a single pen (8 × 4.8 m). Heifers were fed once daily at 0900 h using the Calan gate feeding system (American Calan Inc.) in individual feed tubs to allow measurement of individual feed intakes. Treatments were formulated considering the maximum tolerable salt in diets of dairy cattle which is around 40 g NaCl/kg DM or 1.40 g NaCl/kg BW (NRC, 2001). Our diets provided approximately 29 g NaCl/kg DM.

The feed was mixed and distributed using a motorized feeding vehicle (Data Ranger, American Calan Inc.). Rations were limit-fed at 2.15% of BW (DM basis) to provide 14% CP and

2.26 Mcal ME / kg of DM using NRC (2001). Rations were adjusted every wk based on BW measurements and DM analysis of feed ingredients (Table 2). Heifers were adapted to diets for 14 d followed by 7 d of collection. Heifers had ad libitum access to water through automatic refilling water troughs and were watched daily for any health problem according to the routine management of the research center. Every wk on Wednesday before feeding throughout the study, BW of heifers were recorded using a platform scale (Cardinal, Northeast Scale Co. Inc., Hooksett, NH).

Feed and fecal sampling and analysis

Samples of TMR for each treatment were collected from d 14 to d 17 and stored at -20°C for future nutrient analysis. Individual feed ingredients were sampled for nutrient analysis every 2 wk or any time new silos of grass and corn silages were opened. Fecal samples were collected from d 17 to d 21 for every 12 h interval offset by 3 h on subsequent days to provide representative samples over 24 h period. Rectal fecal grabs samples (~ 200 g/sample) were collected via gloved hand directly from the rectum. Fresh fecal samples over the 4-d of the collection period were combined to obtain a single composite and were frozen at -20°C.

Feed and fecal samples were then thawed at room temperature and emptied into aluminum trays to be dried in a forced-air convection oven (Binder, Bohemia, NY) at 55°C for 48 h (for feed samples) and at least 96h for fecal samples until completely dried. The dried TMR, orts, and fecal samples were ground through a 1-mm screen using a Wiley mill (Model 3, Arthur H. Thomas, Philadelphia, PA). Ground samples were sent to Rock River Laboratory (Watertown, WI) for nutrient analysis.

Samples were analyzed for NDF (method 6 in an Ankom Fiber Analyzer A2000 with α-amylase and sodium sulfite, Ankom Technology; solutions as in Van Soest et al., 1991), and ADF (method 5 in an Ankom Fiber Analyzer A2000, Ankom Technology, Fairpoint, NY; method 973.18, AOAC

International, 1998). Hemicellulose was calculated as NDF% - ADF%. Nitrogen was analyzed via Dumas combustion (AOAC International 2002; method 968.06) on a Rapid N cube (Elementar Analysensystem, GmbH, Hanau Germany). Nitrogen was then multiplied by 6.25 to calculate CP. Starch concentration was analyzed using a modified method of glucose analysis (Bach Knudsen, 1997) completed on a YSI 2700 select Biochemistry Analyzer (YSI Biochemistry analyzer, YSI Inc., Yellow Springs, OH), and crude fat was analyzed with ether extraction technique (method 2003.05; AOAC International, 2006). Ash content was determined by incinerating 1 g of sample for 8 h at 450°C in a muffle furnace (AOAC International, 2002; method 942.05). Mineral composition analysis included Ca, P, Mg, K, Na (AOAC International, 1998; method 985.01), and S (AOAC International, 1998; method 923.01). Acid insoluble ash (AIA)was determined according Van Keulen and Young (1977).

Urine sampling and analysis

Urine samples were collected at the same time as fecal samples, via manual stimulation of the pudendal nerve. Urinary samples were immediately transported to the laboratory where 1 mL subsample of urine was pooled over 4 d into centrifuge tubes containing 32 mL of 0.072 *N* H_2SO4 for later analysis of creatinine, allantoin, and uric acid.

Samples were thawed at room temperature and analyzed calorimetrically for: creatinine (assay kit # 5007001, Cayman Chemical C., Ann Arbor, MI) using a microplate reader (Epoch Bio Tek Instruments, Inc., Winooski, VT) set at a wavelength of 492 nm, allantoin (Chen and Gomes, 1992), and uric acid (Assay kit # 700320, Cayman chemical company., Ann Arbor, MI) using a fluorometer (SpectraMax M2e, Orleans Drive Sunnyvale, CA), set at an excitation wavelength of 530 nm and an emission wavelength of 585. Urinary volume was estimated from sample urinary concentration of creatinine and the creatinine excretion [using a regression equation by Chizzotti

et al. (2008) for growing heifers: Creatinine excretion (CE) /kg BW = 0.28 ± 0.01– 0.000097 ± 0.000015 × BW]. Urinary excretion of total purine derivatives was calculated by adding allantoin and uric acid excretion.

Acid detergent insoluble ash (AIA) was used as an internal digesta marker to estimate 24 h fecal excretion and apparent total-tract nutrient digestibility was determined. Feed and fecal sampling were analyzed for ADF using the filter bag technique (method 5, Ankom Technology) followed by the determination of AIA according to Van Keulen and Young (1977). The equation used to estimate apparent total tract nutrient digestibility was:

$$\text{Apparent total tract nutrient digestibility (\%)} = 100 - 100 \times \frac{\%\ \text{AIA in feed} \times \%\ \text{Nutrient in feces}}{\%\ \text{AIA in feces} \times \%\ \text{Nutrient in feed}}$$

Statistical analysis

Data were analyzed as a replicated 4x4 Latin square design. Initial BW served as a covariate for BW, ADG (average daily gain), and Gain:Feed ratio using the Mixed procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). The model included square, period, heifer and treatment according to the following model: $Y_{ijkl} = \mu + S_i + P_j + H_k + X_{ijk} + T_l + ST_{il} + E_{ijkl}$,

where Y_{ijkl} = the dependent variable, μ = the overall mean, S_i = random effect of square (i = 1, 2), P_j = the random effect of period (j = 1, ...,4), H_k = the random effect of heifer (k = 1, ...,8) , Tk = the fixed effect of the k_{th} treatment (k = 0; 0.8, 1.6 and 2.4% salt in WBG), X_{ijk} = the covariate measurement for BW, ADG and Feed:Gain); ST_{il} = fixed effect of interaction between the i_{th} square and l_{th} treatment; and the E_{ijkl} = the residual error. Degrees of freedom were calculated using the Kenward-Roger approximation option of the Mixed procedure. Single degree of freedom contrasts for linear, quadratic and cubic effects were determined. If the probability of the covariate parameter estimate was greater than 0.25, the covariate was removed from the model. Square × treatment

interaction was analyzed and removed in the model when not significant ($P > 0.05$). For all variables, the least-square means for each treatment were reported. Significant treatment effects were declared at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

The goal of this experiment was to evaluate the apparent total-tract nutrient digestibility and purine derivative excretion in growing Holstein heifers limit-fed with diets containing WBG treated with different amounts of salt. Much research has been conducted to evaluate the effects of different preservation techniques on improving the shelf-life of WBG (Al-Hadith et al., 1985; Santos et al., 2003; Marston et al., 2009; Souza et al., 2016; Hatungimana and Erickson, 2019), however, few of the techniques were evaluated on their effect on nutrient digestibility.

Feed Nutrients and Diet Composition

The average nutrient composition of major feed ingredients used in this study is presented in Table 13. During the study, the DM content of WBG averaged 22.6%, and CP averaged 32.1%. The DM content of WBG varies depending on grain source and processing technology used in brewing. Dhiman et al. (2003) reported the range of the DM content of WBG to be between 25 and 35%, while Frasson et al. (2018) reported the CP content of WBG to range from 17 to 35%. In this study, there was little variation of nutrients for different batches of WBG as it was supplied from the same source for the entire course of the experiment.

Table 14 illustrates the dietary ingredient composition (% DM). Dietary ingredients were similar in all treatments except the level of salt mixed with fresh WBG during storage. Salt was balanced in diets during feeding to equalize the amount of salt consumed by heifers.

Table 15 contains the nutrient composition of diets based on laboratory analysis. The DM and CP contents of diets were similar across treatments. The NDF content was numerically less for diets containing WBG treated with greater amounts of salt (1.6% and 2.4% salt). Starch, and non-fiber carbohydrate (NFC) were numerically greater in heifers fed diets containing WBG treated with greater amounts of salt. The reduced levels of NDF content and greater levels of NFC observed in 1.6% and 2.4% salt treatments were probably due to storing WBG with salt that causes fiber degradation and enhances pectin solubilization (Van Buren, 2006).

Yeast and Mold Growth and Wet Brewer's Grains Losses

The yeast and mold growth counts in treated WBG are presented in Table 16. Yeast and mold CFU were not different among treatments. According to Masui et al. (1979), yeasts are tolerant to high salt concentrations because their membrane lipid bilayers have a high density of negative charges that allow yeasts to counter the high cation concentration (Na+ on the outside and K+ on the inside; Russell, 1989). Hatungimana and Erickson (2019) reported a decrease in mold counts in WBG treated with 2.6 and 3.8% salt. According to Csonka (1989), salt inhibits the growth of mold by plasmolysis which causes the shrinkage of the cytoplasmic volume. Moreover, salt decreases water activity and interrupts vital microbial and enzymatic processes (Lawrence et al., 2003; Albarracin et al., 2011).

The loss of WBG weight (% initial weight) exhibited a linear effect (P = 0.02). As salt amounts increased, WBG loss decreased. Nadi and Shokri (2012), reported that water evaporation rate decreases as NaCl concentration increases to a certain level (1.5 Molar) but any further increase in NaCl concentration may result in higher evaporation rate. While not significantly different, the inhibition of mold growth may have contributed to the reduced WBG losses.

Dry Matter Intake and Growth Performance of Heifers

Table 17 illustrates the BW, ADG and Gain: feed ratio. The BW, ADG and Gain:feed ratio increased linearly ($P = 0.04$, $P = 0.03$ and $P = 0.03$ respectively) with increasing amount of salt in WBG. The BW, ADG, and Gain:feed were greater in heifers fed diets containing WBG treated with salt than the control. According to Harrison et al. (1975) and Amaral et al. (1984), the short residence of digesta in the rumen may increase the efficiency of rumen microbial protein synthesis, as well as bypass of high-quality dietary nutrient such as protein and starch which may have contributed greater growth performance. Croom et al. (1982) observed an increased feed efficiency with diets containing high levels of salt in beef steers and suggested the mechanism behind this effect was via increased rate of passage of dietary nutrients in the rumen. However, the diets used in the current experiment were balanced for salt and we think that treating WBG with salt increased its nutrient digestibility and availability to heifers.

Apparent Total Tract Nutrient Digestibility

The DMI and total-tract nutrient digestibility in heifers limit-fed with diets containing WBG treated with different amounts of salt is presented in Table 18. The DMI exhibited a cubic effect. The DMI was greater in heifers fed 1.6 % and 2.4% salt. According to Ferguson and Solomon (1971), increasing levels of NaCl in the rumen stimulate the animal to consume more water which increases the passage rate of digesta thereby decreasing the residence time of feed. However, greater DMI observed with increasing levels of salt in WBG was probably because heifers were limit-fed based on their BW and less DM loss in WBG treated with salt as a result of mold growth inhibition. It is well known that increasing feeding levels decreases diet digestibility (Huhtanen et al. 2009), however, an associative effect between the feeding level and diet composition would be more important to predict diet digestibility (Tyrrel and Moe, 1975; Colucci

et al., 1982). Moreover, it was reported by Hatungimana and Erickson, (2019) that preserving WBG with salt inhibited the growth of molds that are responsible of spoilage and nutrient losses. In this experiment, mold counts were not different among treatments due probably to the short time of WBG storage.

The digestibility of DM, OM, CP, and NFC decreased linearly with increasing levels of salt in WBG (P = 0.03, P = 0.02, P = 0.04, and P = 0.01, respectively). Crude fat digestibility exhibited a quadratic effect (P = 0.04). The decreased digestibility of DM, CP, and NFC was probably due to the increased passage rate in the rumen due to the increased ruminal dilution rate (Amaral et al., 1985). Treating and storing WBG with salt prevented mold growth which is harmful to ruminal bacteria through mycotoxins production (Hatungimana and Erickson, 2019). Moreover, salt treatment may have caused fiber degradation and pectin solubilization in WBG (Van Buren, 1986) which may have contributed to the shorter residence time of digesta in the rumen. Croom et al. (1982) observed a linear increase in the molar proportion of acetate while that of propionate decreased when salt was added to the diets of steers. Elevated acetate to propionate ratio was found to be correlated with an increased ruminal fluid dilution rate in sheep and cattle (Harrison et al., 1975; Rogers et al., 1975).

Compared to other methods of evaluating digestibility such as in vitro and in situ methods, in vivo total-tract digestibility presents some sources of variation. The increased intake entails an increase in the rate of passage of ingested feed through the gastrointestinal tract which shortens the length of the feed material that is exposed to enzyme actions and results in considerable depression of digestibility (Kitessa et al., 1999). Another source of variation is the associative effect between feeds. When two or more feeds of different digestibility coefficients are fed in

mixture, the digestibility of the whole mixture is different from the mean digestibility coefficient of the feeds in the mixture (Kitessa et al., 1999).

Urinary Volume and Purine Derivative Excretion

Table 19 illustrates least-square means for urinary volume and purine derivative excretion in heifers. The urinary volume was not different among treatments. The fact that heifers consumed the same amount of salt in their diets explains the lack of significant difference in the urinary output. In dairy cows, salt intake leads to increased water intake and urination rate to prevent the surplus of sodium in the body (NRC, 2007b). According to NRC (2001), the maximum level of NaCl in feed for dairy cattle is approximately 40 g NaCl/kg DM. Our diets provided around 29 g of salt/kg DM which was much less than the maximum level to be detrimental to heifers' health.

The output of allantoin, uric acid and total purine derivative (PD) were similar among treatments. In ruminant animals, allantoin and uric acid are excreted as PD in urine and they are related to microbial protein supplied to the host animal. According to Chen and Gomes (1992), there is a linear response curve of PD excreted versus purines absorbed. The microbial nitrogen (MN) yields were reported to range from 14 to 49 g of MN/kg of OM apparently digested in the rumen (Chen et al., 1992). Nangia and Sharma (1994), reported greater microbial protein N synthesis in the rumen of animals fed salt. In this experiment, heifers consumed the same amount of salt and this may be the reason why no difference in PD excretion was observed among treatments.

CONCLUSION

In this study, treating and storing WBG with incremental levels of salt before being included in diets resulted in decreased nutrient digestibility of DM, OM, and CP but not for starch

and crude fat. However, heifers fed diets containing WBG treated with incremental levels of salt had greater BW, ADG and feed efficiency than heifers fed the control diet. While this experiment was not designed to evaluate growth, these effects need to be evaluated in larger feeding studies. Urinary output and PD excretion were not affected by salt treatment especially because total salt consumption was similar for all heifers. Further studies should investigate the effect of direct inclusion of salt in heifers' diets on ruminal fermentation and microbial protein synthesis. Salt treatments did not affect yeast or mold growth in WBG. Moreover, lesser WBG losses were observed in WBG treated with salt compared to the control. On a practical point, dairy producers can use salt (spread salt on top or mix with WBG) to prevent mold growth in WBG.

Table 13**:** Average nutrient composition of major ingredients used in the experiment

Item, % DM	Grass silage	Corn silage	WBG[1]	Corn meal	Soybean meal
DM	33.5 ± 2.9	34.3 ± 0.7	22.6 ± 0.90	86.9	87.6
CP	12.4 ± 2.4	7 ± 0.3	32.1 ± 2.16	8.25	53.3
aNDF	59.07 ± 5.4	42.4 ± 2.6	43.5 ± 8.40	9.8	9.0
ADF	39.7 ± 3.7	25.2 ± 1.8	22.2 ± 3.60	4.25	6.0
Lignin	6.67 ± 0.87	3.3 ± 0.3	11.2 ± 4.40	1.33	0.7
Starch	0.77 ± 0.48	32.8 ± 2.6	3.4 ± 2.7	74.01	8.05
Fat	3.61 ± 0.75	2.98 ± 0.48	8.83 ± 1.07	3.95	1.9
Ash	7.74 ± 1.2	3.49 ± 0.20	4.79 ± 0.24	1.52	6.36
Ca	0.70 ± 0.12	0.18 ± 0.03	0.17 ± 0.01	0.02	0.50
P	0.28 ± 0.03	0.25 ± 0.02	0.59 ± 0.04	0.27	0.65
K	2.35 ± 0.42	1.08 ± 0.05	0.07 ± 0.04	0.39	2.48
Mg	0.22 ± 0.04	0.16 ± 0.005	0.18 ± 0.01	0.10	0.34
S	0.21 ± 0.03	0.10 ± 0.01	0.35 ±0.02	0.11	0.41

[1]WBG = Wet brewer’s grains

Table 14: Ingredient composition (% DM) of experimental diets containing 0, 0.8, 1.6 and 2.4% salt in wet brewer's grains limit- fed to dairy heifers

Ingredient, % DM	Treatments (% salt in WBG)[1]			
	0	0.8	1.6	2.4
Grass silage	9	9	9	9
Corn silage	47	47	47	47
Corn meal	19	19	19	19
Soybean meal	2	2	2	2
Wet brewer's grain	20	20	20	20
Salt[2]	2.4	1.6	0.8	0
Mineral and vitamin mix[3]	3	3	3	3

[1]Treatments: 0% salt = WBG were stored 4 d without addition of salt; 0.8% salt = WBG were mixed and stored 4 d with 0.8% salt. 1.6% salt = WBG were mixed and stored 4 d with 1.6% salt; 2.4% = WBG were mixed and stored 4 d with 2.4% salt.

[2]Salt treatment were added in reverse order to diets during feeding to equalize amounts of salt consumed by heifers.

[3]Contained 19.05 % Ca; 6.01 % P; 3.51 % Mg; 20.00 % Salt; 7.80 % Na; 0. 26 % Fe; 0.26% Zn; 0.26 % Mn; 12.30% Cl; 602 mg/kg Cu; 15.0 mg / kg Co; 25.09 mg/kg Se; and 15.00mg/kg I; 267,800 IU/kg vitamin A; 111,071 IU/kg vitamin D; and 2,207 IU/kg vitamin E.

Table 15: Average nutrient composition of experimental diets containing wet brewer's grains treated with different amounts of salt

	Treatments (% salt in WBG)[1]			
Item %, DM	0%	0.8%	1.6%	2.4%
DM	35.17 ± 1.2	34.3 ± 1.46	35.5 ± 1.19	35.2 ± 1.33
CP	13.7 ± 0.61	13.6 ± 0.82	13.2 ± 0.06	13.4 ± 0.90
aNDF[2]	34.6 ± 0.74	35.1 ± 1.48	32.8 ± 3.12	33.3 ± 1.06
ADF	18.4 ± 0.80	19.0 ± 1.53	18.6 ± 0.90	17.4 ± 1.06
Fat[3]	3.02 ± 0.54	3.12 ± 0.48	3.3 ± 0.85	3.56 ± 0.83
Starch	29.4 ± 1.54	28.9 ± 2.06	31.2 ± 1.14	30.1 ± 1.40
NFC[4]	42.6 ± 1.68	42.0 ± 2.24	44.5 ± 3.2	43.2 ± 1.40
Ash	7.49 ± 0.35	7.57 ± 0.47	7.51 ± 0.20	7.78 ± 0.47
ME[5], Mcal/kg	2.57 ± 0.2	2.57 ± 0.3	2.57 ± 0.2	2.57 ± 0.3

[1]Treatments: 0% salt = WBG were stored 4 d without addition of salt; 0.8% salt = WBG were mixed and stored 4 d with 0.8% salt; 1.6% salt = WBG were mixed and stored 4 d with 1.6% salt. 2.4% = WBG mixed and stored 4 d with 2.4% salt.

[2]α-amylase NDF

[3] Ether extract

[4] NFC, % = 100 – (CP% + (NDF% – neutral detergent insoluble CP%) + fat% + ash%)

[5]Metabolizable energy, estimated from NRC (2001)

Table 16: Yeast and mold growth in wet brewer's grains treated with different amount of salt

Item,	Treatments (% salt in WBG)[1]					Contrast *P*-values		
	0	0.8	1.6	2.4	SEM	L[2]	Q[3]	C[4]
Yeast, log cfu	7.78	8.14	8.33	7.20	0.2	0.69	0.34	0.52
Mold, log cfu	7.00	6.11	6.66	6.76	0.37	0.29	0.19	0.44
WBG weight loss, %	8.82	11.02	5.72	5.12	1.37	0.02	0.30	0.52

[1]Treatments: 0% salt = Wet brewer's grains were mixed and stored 4 d without addition of salt; 0.8% salt = Wet brewer's grains were mixed and stored 4 d with 0.8% salt; 1.6% salt = Wet brewer's grains were mixed and stored 4 d with 1.6% salt; 2.4% = Wet brewer's grains were mixed and stored 4 d with 2.4% salt.

[2]Linear effect

[3]Quadratic effect

[4]Cubic effect

Table 17: Growth performance of heifers limit-fed diets containing wet brewer's grains treated with different amounts of salt

	Treatments (% salt in WBG)[1]					**Contrast *P*-values**		
	0	0.8	1.6	2.4	SEM	L[2]	Q[3]	C[4]
BW, kg	261.16	264.7	265.31	265.21	2.06	0.04	0.17	0.2
ADG, kg/d	0.73	0.88	0.86	1.05	0.09	0.03	0.79	0.08
Gain:Feed	0.14	0.17	0.16	0.21	0.01	0.03	0.78	0.05

[1]Treatments: 0% salt = WBG were stored 4 d without addition of salt; 0.8% salt = WBG were mixed and stored 4 d with 0.8% salt; 1.6% salt = WBG were mixed and stored 4 d with 1.6% salt; 2.4% = WBG were mixed and stored 4 d with 2.4% salt.

[2]Linear effect.

[3]Quadratic effect.

[4]Cubic effect.

Table 18: Total tract nutrient digestibility of heifers limit-fed diets containing wet brewer's grains treated with different amounts of salt

	Treatment (% salt in WBG)[1]					Contrast *P*-values		
Item, %	0	0.8	1.6	2.4	SEM	L[2]	Q[3]	C[4]
DMI, kg/d	5.09	4.97	5.13	5.10	0.01	< 0.01	< 0.01	< 0.01
DM	64.37	61.3	60.2	50.1	4.39	0.03	0.43	0.15
OM	65.5	62.1	61.01	49.68	4.29	0.02	0.37	0.10
CP	56.12	51.46	49.97	41.83	4.69	0.04	0.71	0.19
aNDF[5]	44.53	50.8	35.57	40.9	5.43	0.35	0.93	0.37
ADF	36.8	44.72	30.79	39.05	6.65	0.83	0.97	0.26
Fat[6]	65.3	67.7	74.7	60.6	3.8	0.69	0.04	0.14
Starch	98.9	95.8	98.8	98.5	1.58	0.76	0.39	0.30
NFC[7]	83.9	83.5	81.6	77.57	1.77	0.01	0.27	0.26

[1]Treatments: 0% salt = WBG were stored 4 d without addition of salt. 0.8% salt = WBG were mixed and stored 4 d with 0.8% salt; 1.6% salt = WBG were mixed and stored 4 d with 1.6% salt; 2.4% = WBG were mixed and stored 4 d with 2.4% salt.

[2]Linear effect.

[3]Quadratic effect.

[4]Cubic effect.

[5]α-amylase NDF.

[6]Ether extract.

[7]NFC = 100 – (CP% + (NDF% – neutral detergent insoluble CP%) + fat% + ash%).

Table 19: Purine derivative excretion of heifers limit-fed with diets containing wet brewer's grains treated with different amounts of salt

	Treatments (% salt in WBG)[1]					Contrast *P*-values		
	0	0.8	1.6	2.4	SEM	L[2]	Q[3]	C[4]
Urinary volume, l/d	12.75	18.89	13.38	15.85	2.73	0.75	0.48	0.11
Allantoin, mmol/d	121.22	164.92	133.98	137.80	16.8	0.79	0.21	0.14
Uric acid, mmol/d	4.41	5.59	5.52	5.44	0.58	0.24	0.26	0.32
Total PD, mmol/d	125.63	170.51	139.5	143.24	17.09	0.62	0.21	0.14

[1]Treatments: 0% salt = WBG were mixed and stored 4 d without addition of salt treatment; 0.8% salt = WBG were mixed and stored 4 d with 0.8% salt; 1.6% salt = WBG were mixed and stored 4 d with 1.6% salt; 2.4% salt = WBG were mixed and stored 4 d with 2.4% salt.

[2]Linear effect

[3]Quadratic effect

[4]Cubic effect

REFERENCES

Adenira, H. A., S. H. Abiose, and A. O. Ogunsua. 2008. Production of fungal β-amylase and amyloglucosidase on some Nigerian Agricultural residues. Food Bioprocess Technol. 3: 693-698.

Akins, M. S. 2016. Dairy heifer development and nutrition management. Vet Clin. Food Anim. 32: 303-317.

Alam, M. Z., N. A. Kabbashi., and S. N. Hussin. 2009. Production of bioethanol by direct bioconversion of oil-palm industrial effluent in a stirred-tank bioreactor. J. Ind. Microbiol. Biotechnol. 36: 801-808.

Albarracin, W., I. C. Sanchez, R. Grau, and J. M. Barat. 2011. Salt in food processing; usage and reduction: A review. J. Food Sci. Technol. 46:1329-1336.

Al-Hadithi, A. N., A. A., Muhsen, and A. A. Yaser. 1985. Study on the possibility of using some organic acids as preservatives for brewery by-products. Journal of Agriculture and Water Resources Research. 4:229-242.

Aliyu, S and M. Bala. 2010. Brewer's spent grain: A review of its potentials and applications. Afric. J. Biotechnol. 10:324-331.

Allen, W. R., K. R. Stevenson, and J. B. Smith. 1975. Influence of additives on short-term preservation of wet brewer's grains stored in uncovered piles. Canadian J. Anim. Sci. 55:609-618.

Allen, W. R. and K. R. Stevenson. 1975. Influence of additives on the ensiling process of wet brewers' grains. Can. J. Anim. Sci. 55:391-402.

Alugongo, G. M., J. Xia., Z. Wu., S. Li., Y. wang, and Z. Cao. 2017. Review: Utilization of yeast of Saccharomyces cerevisiae origin in artificially raised calves. J. Anim. Sci. Biotechnol. 8:34.

Amaral, D. M., W. J. Croom., A. H. Rakes., E. S. Leonard, and A. C. Linnerud. 1985. Increased concentration of sodium chloride on milk production of cows fed low fiber diets. J Dairy Sci. 68:2940-2947.

Anderson, J. L., K. F. Kalscheur, J. A. Clapper, G. A. Perry, D. H. Keisler, A. D. Garcia, and D. J. Schingoethe. 2015b. Feeding fat from distillers dried grains with solubles to dairy heifers: II. Effects on metabolic profile. J. Dairy Sci. 98:5709.

Anderson, J. L., K. F. Kalscheur, A. D. Garcia, and D. J. Schingoethe. 2015a. Feeding fat from distillers dried grains with soluble to dairy heifers: Effect on growth performance and total-tract digestibility of nutrients. J. Dairy Sci. 98:5699-5708.

Anderson, J. L., K.F. Kalscheur, A. D. Garcia, D. J. Schingoethe, D.P. Casper, and D.H. Kleinschmit. 2015. Ensiling characteristics of distiller's wet grains with corn stalks and determination of the feeding potential for dairy heifers. Prof. Anim. Sci. 31:359-367.

Ankom Technology, 2017. In vitro True digestibility using the DAISY incubator. Ankom technology 1/24/2017 [Online] Available at http://www.ankom.com/ IVTD-Daisy-PDF.

AOAC International. 1998. Official methods of analysis. 16th edition. AOAC International, Washington, DC.

AOAC International. 1999. Official methods of analysis. 16th ed. AOAC Int., Washington, DC.

AOAC International. 2002. Official methods of analysis. 17th edition. AOAC International, Gaithersburg, MD.

AOAC International. 2006. Official methods of analysis. 18th edition. AOAC International, Gaithersburg, MD.

Aragon, Y. A., 2012. The use of probiotic strains as silage inoculants. Probiotic in animals. DOI: 10.5772/50431.

Armentano, L. E., T. A. Herrington., C. E. Polan., A. J. Moe., J. H. Herbein., and P. Umstadt. 1986. Ruminal degradation of dried, wet brewer's grains and soybean meal. J Dairy Sci. 69:2124-2133.

Bach Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67:319–338. https://doi.org/10.1016/S0377-8401(97)00009-6.

Bal, M. A., J. G. Coors, and R. D. Shaver. 1997. Impact of the maturity of maize for use as silage in the diets of dairy cows on intake, digestion and milk production. J. Dairy Sci. 80:2497-2503.

Balch, C. C., and R. C. Campling. 1962. Regulation of voluntary food intake in ruminants. Nutr. Abstr. Rev. 32:669.

Bannink, A., and V. A. Hindle. 2003. Prediction of N intake and N excretion by dairy cows from milk data. Report 03:0008567 (in Dutch) (Animal Sciences Group, Lelystad, the Netherlands).

Bartolome, B., M. Santos, J. J. Jimenez, M. J. D., Nozal, and C. G. Cordoves. 2002. Pentoses and hydroxycinnamic acids in brewer's spent grain. J. Cereal Sci. 36:51-56.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

Beribasakis, N. G, and D. Tsirogogianni. 1996. Effects of wet brewer's grains on milk yield, milk composition, and blood components of dairy cows in hot weather. An. Feed Sci. Technol. 57:175-181.

Brito, A. F, and G. A. Broderick. 2006. Effect of varying dietary ratios of alfalfa silage to corn silage on production and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 89:3924-3938.

Cai, Y., S. Ohmomo, M. Ogawa, and S. Kumai. 1997. Effect of NaCl-tolerant lactic acid bacteria and NaCl on the fermentation characteristics and aerobic stability of silage. J. Appl. Microbiol. 83:307-313.

Chen, X. B, and M. J. Gomes. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives. An overview of technical details. International Feed Resources Unit, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, UK. Occasional Publication.

Chen, X. B., Chen, Y. K., Franklin., M.F., Orskov, E.R. and Shand, W.J. 1992. The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep. J. Anim. Sci. 70: 1534-1542.

Chiou, P. W. S., C. R. Chen., K. J. Chen, and B. Yu. 1998. Wet brewer's grains or bean curd pomace as partial replacement of soybean meal for lactating cows. Anim. Feed Sci. Technol. 74:123-134.

Chizzotti, M. L., S. C. V. Filho., R. F. D. Valadares., F. H. m. Chizzotti, and L. O. Tedeschi. 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. Livest. Sci. 113: 218-225.

Clark, J. H., M. R. Murphy, and B. A. Crooker. 1987. Supplying the protein needs of dairy cattle from by-product feeds. J. Dairy Sci. 70:1092-1109.

Colucci, P. E., L. E. Chase and P. J., Van Soest. 1982. Feed intake, apparent diet digestibility and rate of particulate passage in dairy cattle. J. Dairy Sci. 65:1445:1456.

Croom, W. J., R. W. Harvey., A. C. Linnerud, and M. Froetschel. 1982. High levels of sodium chloride in beef cattle diets. Can. J. Animal Sci. 62:217-227.

Csonka, L. N. 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev. 53:121-147.

Czerkawski, J. W, and G. Breckenridge. 1997. Design and development of a long-term simulation technique (Rusitec). British Journal of Nutrition. 38:371-389.

Czerkawski, J. W. 1986. An introduction to rumen studies. Pergamon Press, Oxford, UK.

Czerkawski, J. W. 1991. In vitro rumen simulation systems. In: Jouany, J. P (ed.) Rumen microbial metabolism and ruminant digestion. INRA Editions. Paris, 353-355.

Dakowski, P., M. R. Weibsjerg, and T. Hvelplund. 1996. The effect of temperature during processing of rapeseed meal on amino acid degradation in the rumen and digestion in the small intestine. Anim. Feed Sci. Technol. 58: 213-226.

Davis, C. L. and J. K. Drackley. 1998. The development, nutrition, and management of the young calf. Ames. Iowa State University Press.

Davis, C. L., D. A. Grenawalt, and G. C. McCoy. 1983. Feeding Value of Pressed Brewers' Grains for Lactating Dairy Cows. J. Dairy Sci. 66:73-79.

DeNise, S. K., J. D. Robison., G. H. Stott and D. V. Armstrong. 1989. Effects of passive immunity on subsequent production in dairy heifers. J. Dairy Sci. 72:552-554.

Dewhurst, R. J., D. Hepper, and A. Webster. 1995. Comparison of in sacco and in vitro techniques for estimating the rate and extent of rumen fermentation of a range of dietary ingredients. Anim. Feed Sci. Technol. 51:211-229.

Dhiman, T. R., H. R. Bingham, and H. D Radloff. 2003. Production response of lactating fed dried versus wet brewer's grains in diets with similar dry matter. J. Dairy Sci. 86:2914-2921.

Drakley, J. K. 2008. Calf nutrition from birth to breeding. Vet Clin. Food Anim. 24:55-86.

El-Shafey, E. I., M. Gameiro, P. F. M. Correia, and J. Carvalho. 2004. Dewatering of brewers' spent grain using a membrane filter press: a pilot plant study. Separation. Sci. Technol. 39: 3237-3261.

Erasmus, L. J., J. Prinsloo, and P. M. Botha. 1990a. Establishment of a ruminal protein degradation data base for dairy cattle using the in-situ polyester bag technique. 2. Energy source. S. Afr. J. Anim. Sci. 20:124-129.

Erasmus, L. J., J. Prinsloo, and P.M. Botha. 1990b. Establishment of a ruminal protein degradation data base for dairy cattle using the in-situ polyester bag technique. 3. Roughages. S. Afr. J. Anim. Sci. 20:130-135.

Faccenda, A., M. A. Zambom., D. D. Castagnara., A. S. Avila, T. Fernandes, E. I. Eckstein., F. A Anschau, and C. R. Schneider. 2017. Use of dried brewer's grains instead of soybean meal to feed lactating cows. Revista Brasileira de Zootechnica. 46:39-46.

Faichney, G. J. 1993. Digesta flow. In: J. M. Forbes, and J. Frence (Eds.) Quantitative Aspects of Ruminant Digestion and Metabolism. P. 53, C.A.B. Int., Wallingford, UK.

Fastnaught, C. E., 2001. Barley fiber. In: Cho, S. and M. L. Dreher., (Eds), Handbook of Dietary fiber. Marcel Dekker, New York, pp. 519-542.

Ferguson, K. A., and D. H. Solomon. 1971. Method and food composition for feeding ruminants. U.S. patent #3619200.

Firkins, J. L., L. L. Berger, N. R. Merchen and G.C. Fahey, Jr., 1986. Effects of forage particle size, level of feed intake and supplemental protein degradability on microbial protein synthesis and site of nutrient digestion in steers. J. Anim. Sci. 62:1081.

Firkins, J. L., D. I. Haratine, J. T. Sylvester, and M. L. Eastridge. 2002. Lactation performance by dairy cows fed wet brewer's grains or whole cottonseed to replace forage. J. Dairy Sci. 85:2662-2668.

Francisco, E. C. G., M. A. Marsalis, and L. M. Lauriault. 2009. Silage Microbial Inoculants: Use in hot weather conditions. Circular 642. Available at: https://aces.nmsu.edu/pubs/ _circulars/ CR642/

Frasson, M. F., S. C. V. Jaurena., A. M. Menegon., M. M. Severo., J. H. Motta, and W. S. Teixeira. 2018. Intake and performance of lambs finished in feed lot with wet brewer's grains. J. Anim. Sci. Technol. 60:12. https://doi.org/10.1186/s40781-018-0166-8.

Galloway, D. L., A. L. Goetsch, L. A. Forester, A. R. Patial, W. Sun, and Z. B. Johnson. 1993. Digestion, feed intake and live weight gain by cattle consuming bermudagrass hay supplemented with soybean hulls and (or) corn. J. Anim. Sci. 71: 3087-3095.

Gargallo, S., S. Calsamiglia, and A. Ferret. 2006. Technical note: A modified three-step in vitro procedure to determine intestinal digestion of protein. J. Anim. Sci. 84:2163-2167.

Geron, L. J. V., I. L. M. Zeoula, J. A. Erkel, I. N. Prado, E. Bublitz, and O. P. P. Prado. 2010. Consumo, digestibilidade dos nutrientes, produção e composição do leite de vacas alimentadas com resíduo de cervejaria fermentado. Acta Scientiarum. Animal Sciences, Maringá. 32: 69-76.

Hannah, S. M., M. D. Stern, and F. R. Ehle. 1986. Evaluation of dual flow continuous culture system for estimating bacterial fermentation in vivo of mixed diets containing various soay bean products. Anim. Feed Sci. Technol. 16:51-62.

Harrison, D. G., D. E. Beever., D. J. Thomson, and D. F. Osbourn. 1975. Manipulation of rumen fermentation in sheep by increasing the rate of flow of water from the rumen. J. Agric. Sci. Camb. 85: 93-101.

Hasson, H. Z., 1993. High fibre-bread containing brewer's spent grains and its effect on lipid metabolism in rats. Die Nahrung. 37: 576-349.

Hatungimana, E, and P. S. Erickson. 2019. Effects of storage of wet brewer's grains treated with salt or a commercially available preservative on the prevention of spoilage, in vitro and in situ dry matter digestibility and intestinal protein digestibility. Appl. Anim. Sci. 35:464-475.

Heinrichs, A. J, and K. E. Lesmeister. 2005. Rumen development in the dairy calf. In: Garnsworthy, P. C, editor. Calf and heifer rearing. Nottingham (UK): Nottingham University Press. p. 53–65.

Hernandez, A. M., J. L. Rodriguez., B. Lopez., and, O. L. Zerquera. 1999. Caracterización quimicay functional del afrecho de malta. Alimantaria May, 105-107.

Herrington, T. A., L. E. Armentano and, C. E. Polan. 1983. Productive response in lactating cows to diets supplemented with dried brewer's grains or soybean meal. J. Dairy Sci. 66: (suppl. 1), 170.

Hersom, M. J. 2006. By-product feed utilization for forage diets. *In*: 55th Annual Florida Beef Cattle Short Course, Gainesville: University of Florida Institute of Food and Agricultural Sciences. pp 5–14.

Hill, T. M., J. M. Aldrich., and R. L. Schlotterbeck. 2006. Effects of feeding rate of high protein calf milk replacers. Prof. Anim. Sci. 23:649-655.

Hill., T. M., J. M. Aldrich., R. L. Schlotterbeck, and H. G. Bateman. 2006. Effects of feeding rate and concentrations of protein and fat of milk replacers fed to neonatal calves. Prof. Anim. Sci. 22:374-381.

Hoffman, P. C. 1997. Optimum body size of Holstein replacement heifers. J. Anim. Sci. 75:836-845.

Hoffman, P. C., and Kester, K. 2013. Estimating dry matter intake of dairy heifers. Forage focus. University of Wisconsin-Madson. Available at: www.midwestforage.org › pdf › 819.pdf.pdf. Accessed February 4, 2020.

Hoffman, P. C., C. R. Simson, and M. A. Wattiaux. 2007. Limit-feeding of gravid Holstein heifers. Effect on growth, manure nutrient excretion and subsequent early lactation performance. J. Dairy Sci. 90:946-954.

Hoffman, P. C. and L. E. Armentano. 1988. Comparison of brewers wet and dried grains and soybean meal as supplements for dairy cattle. Nutritional Reports International. 38:655-663.

Homm, J. W., Berger, L. L., and T. G. Nash. 2008. Determining the corn replacement value of wet brewer's grain for feedlot heifers. Prof. Anim. Sci. 24:47-51.

Huhtanen, P., M. Rinne, and J. Nousianen. 2009. A meta- analysis of feed digestion in dairy cows. The effects of feeding levels and diet composition on digestibility. J. Dairy Sci. 92:5031-5042.

Huige, N. J. 1994. Brewery by-products and effluents, in: Hardwick, W. A. (Ed), Handbook of Brewing. Marcel Dekker, New York, pp. 501-550.

Imai, A. 2001. Silage making and utilization of high moisture by-products. Grass. Sci. 47: 307–310.

IndexMundi. 2019. Commodity price indices. Available at: https://www.indexmundi.com/commodities/?commodity=soybeans. Accessed at November 14, 2019.

IndexMundi. 2019. Commodity price indices. Available at: https://www.indexmundi.com/commodities/?commodity=corn. Accessed at November 14, 2019.

Johnson, C. O. L. E., J. T. Huber, and K. J. King. 1987. Storage and utilization of brewer's wet grain in diets for lactating dairy cows. J. Dairy Sci. 70:98-107.

Jonsson, A., H. Lindberg, S. Sundas, P. Lingvall, and S. Lindgren. 1990. Effect of additives on quality of big-bale silage. Anim. Feed Sci. Technol. 31:139–155.

Jung, H. G. 1993. Forage cell wall structure and digestibility. American Society of Agronomy, Madson, Wisconsin.

Kaur, V. I, and P. K. Saxena. 2004. Incorporation of brewery waste in supplementary feed and its impact on growth in some carps. Bioresour Technol. 91: 101-104.

Kendal, N.T. 1994. Barley and malt. in: Hardwick, W.A. (Ed.), Handbook of Brewing. Marcel Dekker, New York, pp. 109–120.

Kennedy, P. M. 1985. Influence of cold exposure on digestion of organic matter, rates of passage of digesta n the gastrointestinal tract and feeding and rumination behavior in sheep given four forage diets in the chopped, or ground and pelleted form. British J. Nutr. 53: 159-173.

Khan, M. A., M. U. Nisa, and M. Sarwar. 2003. Techniques of Measuring Digestibility for the Nutritional Evaluation of Feeds. Int. J. Agric. Biol. 5:91-94.

Kissel, L. T, and N. Prentice. 1979. Protein and fiber enrichment of cookies flour with brewer's spent grains. Cereal chemistry. 56: 261-266.

Kitessa, S., P. C. Flinn, and G. G. Irish. 1999. Comparison of methods used to predict the in vivo digestibility of feeds in ruminants. Aus. J. Agric. Res. 50:825-841.

Kleinschmit, D. H., and L. Kung. 2006. A meta-analysis of the effects of Lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. J. Dairy Sci. 89:4005–4013.

Kleinschmit, D. H., J. M. Neylon., T. L. Ebling, J. M. Ladd., J. E. Lynch, and L. Kung. 2002. A comparison of chemical and microbial anti-fungal additives and their effects on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 85 (Suppl. 1): 24.

Kleinschmit, D. H., R. J. Schmidt, and L. Kung. 2005. The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 88:2130-2139.

Kotb, A. R, and T. D. Luckey. 1972. Markers in nutrition. Nutr. Abstr. Rev. 42:813

Kung, L. 2005. Managing high moisture co-product feeds for improved aerobic stability. Proceedings of the four-state dairy Conference, Dubuque, IA. June 15-16.

Kung, L., C. C. Taylor., M. P. Lynch, and J. M. Neylon. 2003. The effect of treating alfalfa with Lactobacillus buchneri 40788 on silage fermentation, aerobic stability, and nutritive value for lactating dairy cows. J. Dairy Sci. 86:336–343.

Kung, L., M. R. Stockes, and C. J. Lin. 2003. Silage additives, In silage Science Technology. In book: Silage Science and Technology, Edition: Agronomy Series 42, Chapter: Silage Additives., Publisher: American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, WS., Editors: D. R. Buxton, R. E. Muck, and J. H. Harrison, pp.305-360.

Kuntzel, U, and H. Sonnerberg. 1997. Preservation of pressed brewer's spent grains with potassium sorbate. Monatsschrift fuer Brauwissesenschaft. 50: 175-181.

Lambert, R. J., and M. Strasford. 1999. Weak acid preservatives. Modeling microbial inhibition and response. J. Appl. Microbiol. 86:157-164.

Lammers, B. P., A. J. Heinrichs, and R. S. Kensinger. 1999. The effects of accelerated growth rates and estrogen implants in prepubertal Holstein heifers on estimates of mammary development and subsequent reproduction and milk production. J. Dairy Sci. 82:1753-64.

Lawrence, R.D., and J.L. Anderson. 2018. Ruminal degradation and intestinal digestibility of camelina meal and carinata meal compared with other protein sources. Prof. Anim. Sci. 34:10-18.

Lawrence, T. E., M. E. Dikeman, J. W. Stephens., E. Obuz, and J. R. Davis. 2003. In situ investigation of the calcium-induced proteolytic and salting-in mechanisms causing tenderization in calcium enhanced muscles. Meat Sci. 66:69-75.

Le Cozler, Y., V. Lollivier., P. Lacasse, and C. Disenhaus. 2008. Rearing strategy and optimizing first-calving targets in dairy heifers: a review. Animal Consortium, 2:9 pp. 1393-1404. Doi:10.101/s1751731108002498.

Li, Q., L. Chai., Z. Yang, and Q. Wang. 2009. Kinetics and thermodynamics of Pb (II) adsorption onto modified spent grain from aqueous solutions. Appl. Surface Sci. 255:4298-4303.

Lilly, V., M. Birch, and B. Garscadden. 1980. The preservation of spent brewer's grains by the application of intermediate moisture food technology. J. Sci. Food Agric. 31: 1059-1065.

Lindberg, J. E., and P.G. Knutsson. 1981. Effect of bag pore size on the loss of particulate matter and on the degradation of cell wall fibre. Agri. Environ. 6: 171-182.

Linko, M., A. Haikara., A. Ritala, and M. Penttila. 1998. Recent advances in the malting and brewing industry. J. Biotechnol. 65: 85–98.

Low, K. S., C. K. Lee., and S. C. Liew. 2000. Sorption of cadmium and lead from aqueous solutions by spent grain. Process. Biochem. 36:39-64.

Lowes, K. F., C. A. Shearman, J. Payne, D. MacKenzie, D. B. Archer, R. J. Merry, and M. J. Gasson. 2000. Prevention of yeast spoilage in feed and food by the yeast mycocin HMK. Appl. Environ. Microbiol. 66:1066–1076.

Lu, S. and S. W. Gibb. 2008. Copper removal from wastewater using spent grain as biosorbent. Bioresour. Technol. 99:1509-1517.

Luginbuhl, J. M., K. R. Pond and J. C. Burns, 1994. Whole tract digesta kinetics and comparison of techniques for the estimation of fecal output in steers fed coastal bermudagrass hay at four levels of intake. J. Anim. Sci. 72: 201-211.

Lynch, K. M., E. J. Steffen, and A. K. Arendt. 2016. Brewer's spent grain: a review with emphasis on food and health. J. Inst. Brew. 122:553-568.

Manthey, A. K., J. L. Anderson, and G. A. Perry. 2016. Feeding distillers dried grains in replacement of forage in limit-fed dairy heifer rations: Effect on growth performance, rumen fermentation, and total-tract digestibility of nutrients. J. Dairy Sci. 99:7206-7215.

Marston S. P., D. A. Spangler, N.L Whitehouse, and P. S. Erickson. 2009. Case study: Addition of a silage preservative reduces spoilage in wet brewer's grain. Appl. Anim. Sci. 25:388-392.

Masui, M., H. Onishi, and T. Unemoto. 1979. Halophilic Microorganisms. pp. 313-348.Tokyo.

McDonald, P., N. Henderson, and S. Heron. 1991. The Biochemistry of Silage. 2nd ed. Chalcombe Publications, Bucks, UK.

McLaughlin, A. M., P. S. Erickson, J. S. Hussey, E. D. Reid, and J. A. Tanguay. 2002. Case Study: NaCl addition to the top layer of corn ensiled in bunker silos. Prof. Anim. Sci. 18:90-92.

Merchen, N. R. 1988. Digestion absorption and excretion in ruminants. In: D. C. Church, (Ed). The Ruminant Animal Digestive Physiology and Nutrition, p. 172. Prentice Hall, Englewood Cliffs, NJ.

Merchen, N., T. Hanson, and T. Klopfenstein. 1979. Ruminal bypass of brewers dried grains protein. J. Anim. Sci. 49: 192-198.

Mertens, D. R. 1993. Kinetics of cell wall digestion and passage in ruminants. USDA-Agricultural Research Service, Madson, Wisconsin.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

Mertens, D. R. 1993. Rate and Extent of digestion. In: Quantitative Aspects of Ruminant Digestion and metabolism. Forbes, J.M., France, J. (Eds.), CAB International University Press, Cambridge, UK, pp. 13-51.

Mertens, D. R. 1994. Regulation of forage intake. p 450- 493. In G. C. Fahey, Jr.(ed) Forage quality, Evaluation and Utilization. ASA, CSSA, Madison, Wisc.

Mills, J. A, and L. Kung. 2002. The effect of delayed ensiling and application of a propionic acid-based additives on the fermentation of barley silage. J. Dairy Sci. 85:1969-1975.

Moallem, U., D. Werner, and H. Lehrer. 2010. Long term effect of ad libitum whole milk and first-lactation milk production. J. Dairy Sci. 93:2639-50.

Ghelichkhan, M., J. S. Eun., R. G. Christensen., R. D. Stott, and J. W. MacAdam. 2018. Urine volume and nitrogen excretion are altered by feeding birdsfoot trefoil compared with alfalfa in lactating dairy cows. J. Anim. Sci. 96:3993-4001.

Moriel, P., L. F. A. Artioli, M. H. Poore, and L. F. Ferraretto. 2015. Dry matter loss and nutritional composition of wet brewer's grains ensiled with or without covering and with or without soybean hulls and propionic acid. Prof. Anim. Sci. 31:559-567.

Mould, F. L., K. E. Kliem, and E. Krystallidou. 2005. A review and simplification of the in vitro incubation medium. Anim. Feed Sci. Technol. 123-124:155-172.

Muck, R. E. 1993. The role of silage additives in making high-quality silage. pp. 106–116. In: Silage production from seed to animal. Proceedings of the National Silage Production Conference. Syracuse, New York, 23-25 February 1993. Ithaca, NY.

Murdock, F. R., Hodgson, A. S and R. E. Riley Jr. 1981. Nutritive value of wet brewer's grains for lactating dairy cows. J. Dairy Sci. 64:1826:1832.

Mussatto, S. I., G. Dragone, and I. C. Roberto. 2006. Brewer's spent: generation, characteristics, and potential applications. J. Cereal Sci. 43: 1-14.

Muthusamy, N. 2014. Chemical composition of brewer's spent grain. A review. Int. J. Sci. Env. Tech. 3: 2109-2112.

Nishino, N., H. Harada, and E. Sagaguchi. 2003. Evaluation of fermentation and aerobic stability of wet brewer's grains ensiled alone or in combination with various feeds as a total mixed ration. J. Sci Food Agric. 83:557-563.

Novik, G. L., J. Wawrzynczyk., O. Norrlow., E. Szwajcer-Dey. 2007. Fractions of barley spent grain as media for the growth of probiotic bacteria. Microbiol. 76: 804-808.

NRC. 2001. Nutrient requirements of Dairy cattle. 7th rev. ed. National Research Council. National Academic Press, Washington DC.

NRC. 2007b. Nutrient requirement of Small Ruminants: sheep, goats, cervids and new world camelids. National Research Council. National Academic Press, Washington DC.

Nwokolo, E. 1986. A comparison of metabolizable energy content of eight common feed ingredients determined with young guinea fowls (keets) and pullet chicks. Anim. Feed. Sci. Technol. 15:1-6.

Okine, E. K, and G.W. Mathison. 1991. External and internal markers for appraising site and extent of digestion in ruminants. J. Dairy Sci. 76:29.

Orskov, E. R. 1982. Protein nutrition in ruminants. Academic Press, London. p. 60

Orskov, E. R., F. D. Hovell, and F. Mould. 1980. The use of nylon bag technique for the evaluation of feedstuffs. Trop Anim. Prod 5: 195-213.

Orskov, E. R., and I. McDonald. 1979. The estimation of rumen degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci., (Camb.) 92:499-503.

Panagiotou, G., P. Granouillet, and P. Olsson. 2006. Of arabinoxylan-degrading enzymes by penicillium brasilianum under solid state fermentation. Appl. Microbiol. Biotechnol. 72: 1117-1124.

Parmenter, R. T., J. W. Rickard, and D. M. James. 2018. Effects of including wet brewer's grains on the growth performance, carcass characteristics and meat quality of finishing steers. Prof. Anim. Sci. 34:505-512. https://doi.org/10.15232/pas.2018-01725.

Payne, M. J., M. N. MacLeod, and R. F. M. Dekker. 1972. Digestion of the dry matter, nitrogen, phosphorous, sulfur, calcium, and detergent fiber fractions of the seeds and pods of stylosanthes humilius contained in terylene bags in the bovine rumen. J. Science Food and Agric. 23: 925-932.

Pereira, J. C., M. D. Carro., J. Gonzalez., M. R. Alvir, and C. A. Rodriguez. 1998. Rumen degradability and intestinal digestibility of brewer's grains as affected by origin and heat treatment and barley rootlets. Anim. Feed Sci. Technol. 74: 107-121.

Pino, F., L. K. Mitchell., C. M. Jones and A. J. Heinrichs. 2018. Comparison of diet digestibility, rumen fermentation, rumen rate of passage, and feed efficiency in dairy heifers fed ad libitum versus precision diets with low- and high-quality forages. J. Applied Anim. Res, 46:1296-1306.

Prentice, N, and B. L. D'Appolonia. 1977. High-fiber bread containing brewer's spent grain. Cereal Chemistry 54, 30-32.

Queiroz, O. C. M., I. M. Ogunade., Z. Weimberg, and A. T. Adesogan. 2018. Silage review. Foodborne pathogens in silage and their mitigation by silage additives. J. Dairy Sci. 101:4132-4142.

Rad, M. N., and N. Shokri. 2012. Nonlinear effects of salt concentrations on evaporation from porous media. Geophysical research letters. 39: L04403. doi:10.1029/2011GL050763.

Ratner, B., H. C. Jackson, and H. L. Gruehl. 1927. Transmission of protein hypersensitiveness from mother to offspring: I. Critique of placenta permeability. J. Immunol. 14:249-265.

Razavi-Rohani, S. M., and M. W. Griffiths. 1999. Antifungal effects of sorbic acid and propionic acid at different pH and NaCl conditions. J. Food Safety. 19:109-120.

Reinold, M. R. 1997. Manual práctico de cervejaria, first ed. Aden Editora e Comunicacões Ltda, São Paulo. 214 p.

Robertson, J. A. I., K. J. A. Anson., J. Treimo., C. B. Faulds., T. F. Brocklehurst., V. G. H. Eijsink, and K. W. Waldron. 2010. Profiling brewer's spent grain for composition and microbial ecology at the site of production. LWT-Food Sci. Technol. 43:890-896.

Robson, J. D., G. H. Stott, and S. K. Denise. 1988. Effects of passive immunity on growth and survival of the dairy heifer. J. Dairy Sci. 71:1283-1287.

Rogers, J. A., C. L. Davis, and J. H. Clark. 1982. Alteration of rumen fermentation, milk fat synthesis, and nutrient utilization with mineral salts in dairy cows. J. Dairy Sci. 65:577–586.

Rogers, J. A., B. C. Marks., C. L. Davis., and J. H. Clark. 1979. Alteration of rumen fermentation in steers by increasing rumen fluid dilution rate with mineral salts. J. Dairy Sci. 62: 1599-1605.

Rooke J. A. 2004. Acetate silages: microbiology and chemistry. Landbauforschung Voelkenrode Sonderheft 123, 309-312. Schroeder, J. W. (2004): Silage fermentation and preservation. NDSU Extension Service, North Dakota State University.

Rotz, C. A., and R. E. Muck. 1994. Changes in forage quality during harvest and storage. In: Fahey, G. C., M. Collins., D. R. Mertens, and L. E. Moser. Forage quality, evaluation and utilization. Madison: ASA, CSSA; SSSA. pp. 828-868.

Rounds., T. Klopfenstein. 1975. Brewers dried grains in ruminant rations. J. Anim. Sci. 41 (Suppl: 1): 415 (Abs.).

Russ, W., H. Mortel, and R. Meyer-Pittroff. 2005. Application of spent grains to increase porosity in bricks. Construction and Building Material. 19:117-126.

Russell, N. J. 1989. Adaptive modifications in membranes of halotolerant and halophilic microorganisms. J. Bioenergetics and Biomembranes. 21:93-113.

Santos, M., J. J. Jimenez., B. Baltolomé., C. Gomez-Cordovés, and M. J. del Nozal. 2003. Variability of brewer's spent grains within a brewery. Food Chemistry. 80: 17-21.

Sarwar, M., C.S. Ali. and M. Z. Alam. 1992. Ruminal degradation of sodium hydroxide treated cellulose material. Pakistan Vet. J. 12: 75.

Sarwar, M., M. A. Iqbal, C. S. Ali, and T. Khaliq. 1994. Growth performance of buffalo male calves as affected by using cowpeas and soybean seeds as sources of urease during urea treated wheat straw ensiling process. Egyptian J. Anim. Prod. 31: 179.

Schingoethe, D. J., F. M. Buyers, and G. T. Schelling. 1988. Nutrient needs during critical periods of the life cycle. The Ruminant Animal: Digestive Physiology and Nutrition. Ed. D. C. Church. New Jersey: Prentice-Hall, Inc. 421-447.

Schneider, B. H, and W. P. Flat. 1975. The Evaluation of Feeds Through Digestibility Experiments. The University of Georgia Press, Athens, GA.

Schneider, P. L., D. K. Beede, and C. J. Wilcox. 1988. Effects of supplemental potassium and sodium chloride salt on ruminal turnover rates, acid-base and mineral status of lactating dairy cows during heat stress. J. Anim. Sci. 66:126-135.

Schneider R. M., J. H. Harrison and K. A. Loney. 1995. The effect of bacteria inoculant, beet pulp, and propionic acid on ensiled wet brewer's grains. J. Dairy Sci. 78:1096-1105.

Seymour, W. M., and C. E. Polan. 1986. Dietary energy regulation during gestation on subsequent lactational response to soybean meal or dried brewer's grains. J. Dairy Sci. 77:2318-2331.

Shand, P. J., J. J. McKinnon., and D. A. Christensen. 1998. Eating quality of beef from animals fed wet brewer's grains and wheat-based wet distiller's grains. Can. J. Anim. Sci. 78:143-146.

Shockey, W. L, and D. C. Borger. 1991. Effect of salt on fermentation of Alfalfa. 2. Treatment of sodium chloride, *Clostridium butyricum*, and lactic acid bacteria. J. Dairy Sci. 74:160-166.

Souza, L. C., M. A. Zambom., M. S. S. Pozza., M. A. N. Neres., A. C. Radis., L. Borsatti., D. D. C. Castagnara, and S. Gundit. 2012. Development of microorganisms during storage of wet brewery waste under aerobic and anaerobic conditions. Revista Brasileira de Zootecnia., v.41, n.1, p.188-193. State Dairy Nutrition Conference, Fort Wayne, IN.

Speck, J. W., A. Bannink, G. Gort, W. H. Hendriks, and J. Dijkstra. 2012. Effect of sodium chloride intake on urine volume, urinary urea excretion, and milk urea concentration in lactating dairy cattle. J. Dairy Sci. 95:7288-7298.

Stojceska, V, and P. Ainsworth. 2008. The effect of different enzymes on the quality of high-fiber enriched brewer's spent grain breads. Food Chem. 110: 865-872.

Stott, G. H., D. B., D. B. Marx., B. E. Menefee, and G. T. Nightengale. 1979. Colostral immunoglobulin transfer in calves. I: Period of absorption. J. Dairy Sci. 62:1632-1638.

Szponar, B., K. J. Pawlik., A. Gamian., and E. S. Dey. 2003. Protein fraction of barley spent grain as a new simple medium for growth and sporulation of soil actinobacteria. Biotechnology Letters. 25:1717-1721.

Tamminga, M. K, and B. A. Williams. 1998. In vitro techniques as tools to predict nutrient supply in ruminants. In: Deaville, E. R., E. Owen., A. T. Adesogan., C. Rymer., J. A. Hungton, and T. L. J. Lawrence (eds) In vitro techniques for measuring nutrient supply to ruminants, Occasional publication No. 22. British Society of animal Science, Edinburgh, pp. 1-11.

Tang, D., G. Yin., Y. He., S. Hu., B. Li., L. Li., H. Liang, and D. Borthakur. 2009. Recovery of protein from brewer’s spent grain by ultrafiltration. Biochem. Eng. J. 48: 1-5.

Tang, Z., S. Cenkowiski, and M. E. Muir. 2004. Modeling the superheated steam-drying of a fixed bed of brewer's spent grain. Biosystems Engineering. 87: 67-77.

Taormina, P. J. 2010. Implications of salt and sodium reduction on microbial food safety. Critical reviews in Food Science and Nutrition. 50:209-227.

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of the forage crops. J. Brit. Grassland Soc. 18:104-111.

Tozer, P. R., and A. J. Heinrichs. 2001. What affects the costs of raising replacement dairy heifers: A multiple-components analysis. J. Dairy Sci. 84: 1836-1844.

Tyrrel, H. F., and P. W. Moe. 1975. Effect of intake on digestive efficiency. J. Dairy Sci. 58:1151–1163.

Van Buren, J. P. 2006. Snap bean texture softening and pectin solubilization caused by the presence of salt during cooking. J. Food Sci. 51: 131-134.

Van Keulen, J. and B. A. Young. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Sci. 44:282-287.

Van Soest, P. J. 1994. The nutritional ecology of the ruminant. 2nd edition, Cornell Univ Press, Ithaca, p. 476.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for Dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Vietor, R. J., A. G. J. Voragen, and S. A. G. F. Angelino. 1993. Composition of non-starch polysaccharides in wort and spent grain from brewing trial with malt good quality barley and a feed barley. J. Inst. Brew. 99:243-248.

Waller, J., N. Merchen, T. Hanson, and T. Klopfenstein. 1980. Effect of sampling intervals and digesta markers on abnormal flow determinations. J. Anim. Sci. 50: 1122-1126.

Wang, B., Y. Luo., K. H. Myung, and J. X. Liu. 2014. Effect of storage duration and temperature on the chemical composition, microorganism density, and in vitro rumen fermentation of wet brewer's grains. Asian Australas. J. Anim. Sci. 27: 832-840.

Weiss, W. P. 1994. Estimation of digestibility of forages by laboratory methods. In Proceedings of the National Conference on Forage quality, Evaluation and utilization. Fahey Jr., G.C., Moser, L.E., Mertens, D.R., Collins, M. (Eds.), University of Nebraska, pp. 664-681.

Wenge, J., I. Steinhofel., C. Henrich, M. Coenen, and L. Bachmann. 2014. Water and concentrate intake, weight gain and duration of diarrhea in young suckling calves on different diets. Livest. Sci. 159:133-140.

West, J. W., L. O. Ely, and S. A. Martin. 1994. Wet brewer's grains for lactating dairy cows during hot, humid weather. J. Dairy Sci. 77:196-204.

Westendorf, M. L., and J. E. Wohlt. 2002. Brewing by-products: their use as animal feeds. Vet. Clin. Food Anim. 18:233-252.

Westendorf, M. L., J. E. Wohlt, C. J. Sniffen, and R. T. Ward. 2014. Nutrient content of brewer's grains at a commercial brewery: Variation in protein, fiber, carbohydrate, fat, and minerals. Prof. Anim. Sci. 30:400-406.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt, and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495-501.

Woolford, M. K. 1975. Microbiological screening of straight-chain fatty acids (C1–C12) as potential silage additives. J. Sci. Food Agric. 26:219–228.

Woolford, M. K. 1984. The silage fermentation. Microbiol. Ser. 14. Marcel Dekker, New York.

Woonton, B. W., J. V. Jacobsen., F. Sherkat and I. M. Stuart. 2005. Change in germination and malting quality during storage of barley. J. Inst. Brew. 111:33-41.

Xiros, C., E. Topakas., P. Katapodis., and P. Christakopoulos. 2008. Hydrolysis and fermentation of brewer's spent grain by Neurospora crassa. Bioresour. Technol. 99:5427:5435.

Zanton, G. I., and A. J. Heinrichs. 2005. A meta-analysis to assess effect of prepubertal average daily gain of Holstein heifers on first-lactation production. J. Dairy Sci. 88:3860-3867.

Zanton, G. I, and A. J. Heinrichs. 2007. Review: Limit-feeding with altered forage-to-concentrate levels in dairy heifer diets. Prof. Anim. Sci. 25:393-403.

Zanton, G. I., and A. J. Heinrichs. 2007. The effects of controlled feeding of a high-forage or high-concentrate ration on heifer growth and first-lactation milk production. J. Dairy Sci. 90:3388-3396.

Zanton, G. I, and J. Heinrichs. 2008. Precision feeding dairy heifers: Strategies and recommendations. Pennsylvania State Univ. DAS-08–130. Pennsylvania State Univ., University Park.

Zanton, G. I, and A. J. Heinrichs. 2009. Limit-feeding with altered forage-to-concentrate levels in dairy heifers' diets. Appl. Anim. Sci. 25:393-403.

Zanton, G. I, and J. Heinrichs. 2016. Precision feeding dairy heifers: Updated: Strategies and recommendations. Pennsylvania State Univ. DAS-08–130. Pennsylvania State Univ., University Park.

APPENDICES

Appendix A IACUC Approval Form Chapter II

University *of* New Hampshire

Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

24-Jul-2017

Erickson, Peter S
Biological Sciences
Dairy Nutrition Res Ctr
Durham, NH 03824-3536

IACUC #: 170705
Project: Total Tract Digestibility of Wet Brewer's Grains Treated with a Commercial Preservative Silo-King GPX and Salt
Approval Date: 20-Jul-2017

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category C on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics or other assessments.* The IACUC made the following comment(s) on this protocol:

1. *Per the investigator, the IACUC changed the Anticipated Completion Date to September 15, 2018.*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jill A. McGaughy, Ph.D.
Chair

Appendix B IACUC Approval Form Chapter III

University *of* New Hampshire

Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

24-Jul-2017

Erickson, Peter S
Biological Sciences
Dairy Nutrition Res Ctr
Durham, NH 03824-3536

IACUC #: 170603
Project: Effect of Partial Replacement of Corn and Soybean Meal by Wet Brewers' Grains on Growth Performance of Dairy Heifers
Approval Date: 20-Jul-2017

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category C on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics or other assessments.* The IACUC made the following comment(s) on this protocol:

1. *As this is a feed study, the IACUC changed the pain and distress category from B to C.*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

Appendix C IACUC Approval Form Chapter IV

University *of* New Hampshire

Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

21-Jun-2018

Erickson, Peter S
Agriculture, Nutrition, & Food Systems
Keener Dairy Research Building
Durham, NH 03824

IACUC #: 180506
Project: Effect of Treating Wet Brewer's Grains wit Salt on in vivo Nutrient Total-tract Digestibility of Dairy Heifers' Diet
Approval Date: 19-Jun-2018

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under pain or distress category C - *the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics o other assessments.* The IACUC made the following comment(s) on this protocol:

1. *Per the discussion at the meeting, the IACUC changed the pain or distress category from "B" to "C and corrected the percentage typographical errors on page 3 and on page 13 of the application.*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of t original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alik Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003

For the IACUC,
Jessica Boker
Jessica A. Boker, Ph.D.
Chair